Studies on Pharmacognosy, Phytochemistry and Antimicrobial Activity of Indian Medicinal Plant Drug

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ABSTRACT

The present study investigated the pharmacognostic and phytochemical profiles of selected Indian medicinal plant drug to explore its therapeutic potential and antimicrobial properties. Comprehensive pharmacognostic evaluations, including macroscopic and microscopic analyses, were conducted to authenticate and standardize the plant material. Phytochemical screening revealed the presence of key bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and glycosides. The antimicrobial efficacy of the selected plant extracts of Capparis decidua was evaluated against a panel of pathogenic microorganisms, including Gram-positive and Gram-negative bacteria and fungi, using the agar well diffusion and microdilution methods. Results demonstrated significant antimicrobial activity, with minimum inhibitory concentrations (MICs) ranging between 50 and 500 µg/mL for select extracts. These findings highlight the potential of these Indian medicinal plants as sources of bioactive compounds for developing novel antimicrobial agents. The study underscores the importance of integrating pharmacognosy and phytochemistry with microbiological assessments to validate the traditional medicinal uses of plants and guide future drug discovery efforts.

Keywords: *Pharmacognostic, phytochemical, minimum inhibitory concentrations, antimicrobial, herbal drug*

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1. Introduction

Plant-derived medicines have been an integral part of traditional healthcare systems around the world for centuries, serving as a diverse source of bioactive molecules with the potential to treat a wide range of diseases. With its high biodiversity and millennia of Ayurvedic, Siddha, and Unani practices, plants are deeply embedded in the health care system in India. Pharmacognosy and phytochemistry are the fields of study that have helped researchers in systematically studying these plants, so there is a link between traditional knowledge and pharmacological validation.[1,2]

Pharmacognosy, the study of natural drugs, focuses on the identification, authentication and quality control of plant-based materials. Phytochemistry fills in the details by looking at the chemical principles underlying a plant's ability to have therapeutic effects. These disciplines combined facilitate the investigation of medicinal plants to identify chemical substances that can serve as new bioactive compounds to be utilized as drugs, especially against infectious diseases. The rising threat of antimicrobial resistance (AMR), has increased the urgency of identifying new therapeutic agents. Recent decades of indiscriminate use of synthetic antibiotics left most of the bacterial and fungal pathogens with acquired resistance to conventional drugs and driven to more morbidity and mortality around the globe. One suitable alternative are medicinal plants that synthesize plant secondary metabolites, such as alkaloids, flavonoids, tannins and phenolics, with effective antimicrobial activities. Such bioactive compounds — frequently operating via novel mechanisms of actions — have proven effective against multidrug-resistant pathogens and are thus invaluable leads for new classes of antimicrobial agents going forward.[3,4]

Plants like *Capparis decidua*, *Azadirachta indica*, *Curcuma longa*, *Ocimum sanctum* and *Tinospora cordifolia* are widely used in traditional medicine in India for their antimicrobial, anti-inflammatory and immunomodulatory effect. Nevertheless, even though the medicinal usage of such plants is already well established in ancient texts, a true scientific validation of their pharmacological properties is still emerging. There is a need for extensive studies combining pharmacognostic studies with phytochemical screening and anti-microbial investigations to validate their effectiveness and safety in modern medicine.[5] This present study investigated the pharmacognostic and phytochemical features of one Indian medicinal plant i.e., *Capparis decidua* and reports its antimicrobial activity against some clinically important bacterial and fungal pathogens. The present study not only highlights the significance of pharmacognosy and phytochemistry in drug discovery but also the pivotal role of this medicinal plant in solving modern-day health problems. These results are likely to lead to the design of green, economical and effective antimicrobial agents that could accompany established treatments and help alleviate the increasing burden of drug-resistant infections.

2. Material and Methods

The fresh young and mature stems of *Capparis decidua* were collected at Chouksey Engineering College Bilaspur Chhattisgarh, during flowering season. The authenticity was established by comparing its morphological characters with the available literature and further study.

2.1 Preparation of Samples

Fresh stems of *C. decidua* were washed to remove soil, mud and other adhering material and dried at room temperature under shade. Powder was prepared from the dried samples and stored properly in airtight containers.

2.2 Macroscopical and Microscopical Studies

The stem and its powdered material were studied for its detailed macroscopical and microscopical characters. Free-hand transverse sections of fresh young and old stems were taken and studied. All the observations of the microscopical study were made and recorded with the help of special CCD (charged coupled device, Lawrence and Mayo) camera attached with Olympus magnus inclined trinocular research microscope.[6]

2.3 Determination of Physico-chemical Parameters

Various physicochemical parameters were studied for the stems of C. decidua.[7-9]

2.3.1 Determination of loss on drying

Accurately weighed 10 g of drug (without preliminary drying and cut in parts of about 3 mm thickness) was placed in a tared evaporating dish. The drug was then dried at 105°C for 5 h and weighed. Drying and weighing was continued at one hour interval until difference between two successive weighing corresponded to not more than 0.25%. Constant weight was considered to reach when two successive weighing after drying for 30 min and cooling for 30 min in a desiccator, show not more than 0.01 g difference.

2.3.2 Determination of ash values

Ash values of powder of *C. decidua* stems were determined by the following method:

2.3.2.1 Determination of total ash

About 2 g of accurately weighed stem powder was incinerated in a crucible (tared silica dish) at a temperature not exceeding 450°C in a muffle furnace until it was free from carbon, and then cooled

and weighed. If carbon-free ash could not be obtained in this manner, the charred mass was exhausted with about 2 ml of hot water or a saturated solution of ammonium nitrate, the residue was collected on an ashless filter paper, dried and then ignited to a constant weight. The ash thus obtained was then cooled, weighed and percentage of ash was calculated about the air-dried powdered drug.

2.3.2.2 Determination of acid insoluble ash

The ash obtained from above procedure was boiled for 5 min with 25 ml of dilute (70 g/l) hydrochloric acid and filtered using an ashless filter paper to collect insoluble matter. The ash obtained was washed with hot water and ignited to a constant weight along with the filter paper in a muffle furnace at 450°C. The percentage of acid-insoluble ash was calculated about the air-dried powdered drug.

2.3.2.3 Determination of water-soluble ash

Total ash was boiled for 5 min with 25 ml of water and insoluble matter was collected on an ashless filter paper. It was washed with hot water and ignited for 15 min at temperature not exceeding 450°C in a muffle furnace. Difference in weight of ash and weight of water insoluble matter gave the weight of water-soluble ash. The percentage of water-soluble ash was calculated about the air-dried powdered drug.

2.3.2.4 Determination of extractive values

Extractive values of powder of *C. decidua* stems were determined by the following method:

2.1.1.1 Determination of water-soluble extractive (Hot extraction)

About 4 g of the powdered material was macerated in 100 ml of water in a closed flask for 1 h and was shaken frequently. It was then boiled gently for 1 h on water bath, cooled, weighed and the weight was readjusted. 25 ml of the filtrate was evaporated to dryness in a porcelain dish and dried at 105°C to a constant weight. The percentage of water-soluble extractive was calculated with reference to the air-dried powered drug.

2.1.1.2 Determination of alcohol soluble extractive (Cold extraction)

About 4 g of the air-dried powdered material was macerated with 100 ml of alcohol in a closed flask for 6 h, shaking frequently at an interval of 1 h. It was then allowed to stand for 18 h and filtered rapidly to prevent any loss during evaporation. 25 ml of the filtrate was evaporated to

dryness in a porcelain dish and dried at 105°C to a constant weight. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried powdered drug.

2.3.2.7 Determination of Microbiological Parameters

The microbiological parameters like Total Plate Count, Yeast and Mould Count and E. coli were investigated in *C. decidua* stem powder. Total Plate Count was determined by using horizontal method for the enumeration of microorganisms by colony count technique at 30°C. Yeast and Mould Count was determined by using colony count technique at 25°C, while E. coli was determined by using colony count technique at 37°C.

2.4 Preliminary Phytochemical Screening

The dried powder of stem was subjected to the preliminary phytochemical analysis for the presence of various phytoconstituents like alkaloids, flavonoids, saponins, sterols and terpenoids, carbohydrates, coumarins, tannins and phenolic compounds and glycosides.

2.4.1 Test for alkaloids (Test with Dragendorff's reagent)

1 g of powder was extracted with 20 ml alcohol (95%) by refluxing for 15 min and filtered and the filtrate was evaporated to dryness. The residue was dissolved in 15 ml of 2N H_2SO_4 and filtered. After making alkaline, the filtrate was extracted with chloroform. The residue left after evaporation was tested for the presence of alkaloids with Dragendorff's reagent. Development of orange colored precipitates indicated the presence of alkaloids.

2.4.2 Tests for Flavonoids [10,11]

2.4.2.1 Shinoda test

1 g powdered stem was extracted with 10 ml of ethanol (95% v/v) for 15 min on a boiling water bath and filtered. To the filtrate, a small piece of magnesium ribbon and 3 to 4 drops of concentrated sulphuric acid were added. Development of red color indicated the presence of flavanones.

2.4.2.2 Fluorescence test

1 g powder was extracted with 15 ml methanol for 2 min on a boiling water bath, filtered while hot and evaporated to dryness. To the residue, 0.3 ml of 3% w/v boric acid solution and 1 ml 10% w/v oxalic acid solution were added. The mixture was evaporated to dryness and the residue was

dissolved in 10 ml ether. The fluorescence in the ethereal layer was observed under U.V. light. Development of greenish fluorescence in ethereal layer indicated the presence of flavonoids.

2.4.3 Tests for Phenolic compounds

2.4.3.1 Test with FeCl3

1 g powder of stems was extracted with 10 ml of methanol for 15 min on a boiling water bath and filtered. To the filtrate, a drop of freshly prepared $FeCl_3$ solution was added. Development of brownish green color indicated the presence of phenolics.

2.4.4 Tests for tannins

Aqueous extract of stem was prepared by refluxing 10 g of powdered stem with 50 ml of water for about 1 hour on water bath and was used for the following tests:

2.4.4.1 Test with gelatin

To 2 to 3 ml of aqueous extract, 1% w/w gelatin solution containing sodium chloride was added. Formation of heavy white precipitates indicated the presence of tannins.

2.4.5 Test for saponins

Froth test 0.1 g of powder was vigorously shaken with 5 ml of distilled water in a test tube for 30 sec and was left undisturbed for 20 min. Persistent froth indicated the presence of saponins.

2.5 Estimation of Phytoconstituents

2.5.1 Estimation of total alkaloids

2.5.1.1 Preparation of extract

10 g coarse powder was extracted with 25 ml of 2% v/v ethanolic acetic acid at 100°C for 10 min. The procedure was repeated 2 times. The extracts were mixed and diluted to 100 ml with 2% v/v ethanolic acetic acid respectively.

2.5.1.2 Procedure for calibration curve

The calibration curve was obtained with Bismuth nitrate pentahydrate stock solution (0.01 %w/v). Series dilutions of the stock solution were made by pipetting out 1, 2, 3, 4, 5, 6, 7, 8 and 9 ml stock solution into separate 10 ml standard flasks and diluting upto the volume with distilled water. To

1 ml of this solution, 5 ml of 3% w/v thiourea solution was added to it. The absorbance value of the solution was measured at 435 nm against blank.

2.5.1.3 Procedure for assay of Alkaloids

5 ml sample extract was taken and the pH was maintained at 2 to 2.5 with dilute HCl. To it, 2 ml of 1% v/v Dragendorff's reagent (DR) was added and the precipitates formed were centrifuged. The centrifugate was checked for complete precipitation by adding DR. The precipitates were then washed with alcohol. The filtrate was discarded and the residue was treated with 2 ml 1% w/v disodium sulfide solution. The brownish-black precipitates formed were dissolved in 2 ml concentrated Nitric acid with warming. This solution was diluted to 10 ml in a standard flask with distilled water. To 1 ml of the resultant solution, 5 ml 3% w/v thiourea solution was added. The absorbance was measured at 435 nm against the blank containing nitric acid and thiourea. The factor is obtained from the standard curve, which is a constant for different concentrations.

2.5.1.4 Preparation of Test Extracts for Pharmacological Studies

Three extracts, viz. aqueous extract, alcoholic extract and hydroalcoholic extract of the stem were prepared for the activity. Aqueous extract was prepared by refluxing 100 g of the air-dried powdered stem on water bath with 500 ml of distilled water for 2 h. After filtration, the resulting solution was evaporated under reduced pressure to yield a solid extract which was named as Extract-A. Alcoholic extract was prepared by exhaustive extraction of 100 g of the powdered stem with 500 ml of absolute alcohol for 5 h, using a Soxhlet apparatus. After filtration, the solvent was evaporated under reduced pressure to yield a semi-solid extract which was named as Extract-B. Hydroalcoholic extract was prepared by refluxing 100 g of the powdered stem on water bath with 50% of hydroalcoholic solution for 2 h. After filtration, the resulting solution was evaporated under reduced pressure to yield a semi-solid extract which was named as Extract-C. Extracts - A, B and C were suspended in distilled water using acacia (1%) expressed as mg of extract/kg body weight.

2.6 Screening of Anti-microbial Activity [12,13]

2.6.1 Preparation of test extracts

For the bioassay, all the three extracts, Extracts - A, B and C (prepared according to method mentioned in above section) were dissolved in DMSO to attain a concentration of 100 mg/ml.

2.6.2 Preparation of standard drugs

Ciprofloxacin and Griseofulvin were used as standard antibiotics for bacteria and fungi, respectively. They were dissolved in DMSO to attain a concentration of 100 mg/ml.

2.6.3 Microorganisms for anti-microbial screening

All three extracts, viz. aqueous extract, alcoholic extract and hydro-alcoholic extract of the stem were screened against 9 bacterial strains and 2 fungal strains, which were obtained from Chouksey Engineering College Bilaspur, Chhattisgarh. The test organisms were Gram-positive organisms - *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 9410) and *Enterococcus faecalis* (MTCC 9845); Gram-negative organisms -*Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 7162), *Pseudomonas aeruginosa* (MTCC 1688), *Proteus vulgaris* (MTCC 7299) and *Erwinia carotovora* (MTCC 2760); and Fungal strains - *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 282).

2.6.4 Preparation of media

2.6.4.1 Mueller Hinton Agar Broth: It was prepared by dissolving 21 g of Mueller Hinton Broth (Himedia, Mumbai) in 1000 ml of distilled water. It was sterilized by autoclaving, mixed well and then poured into test tubes for bacterial strains.

2.6.4.2 Mueller Hinton Agar Medium (MHA): The medium was prepared by dissolving 38 g of Mueller Hinton Agar (Himedia, Mumbai) in 1000 ml of distilled water. The dissolved medium was autoclaved. The autoclaved medium was mixed well and poured onto 100 mm sterile Petri plates (25 to 30 ml/plate) while still molten (45°C). This medium was used for the bacterial strains.

2.6.4.3 Sabouraud Dextrose Broth: It was prepared by dissolving 30 g of Sabouraud Dextrose Broth (Himedia, Mumbai) in 1000 ml of distilled water. It was sterilized by autoclaving, mixed well and then poured into test tubes for fungal strains.

2.6.4.4 Sabouraud Dextrose Agar Medium (SDA): 65 g of Sabouraud Dextrose Agar (Himedia, Mumbai) was suspended in 1000 ml of distilled water by boiling. The medium was heated to boiling to dissolve the medium completely. The dissolved medium was autoclaved and then mixed well and poured onto 100 mm Petri plates (25 to 30 ml/plate) while still molten. This nutrient medium was used for the fungal strains.

2.6.5 Preparation of inoculum

Stock cultures were maintained at 4°C on slopes of respective media. Active cultures for experiments were prepared by transferring a loopful of stock cultures to test tubes of Mueller Hinton Broth for bacteria and Sabouraud Dextrose Broth for fungal strains, which were then incubated without agitation for 24 h at 37°C for bacterial strains and 72 h at room temperature for

fungi. The cultures were diluted with respective media to obtain suspensions (100 µl) containing 108 cfu/ml bacteria and 104 spores/ml fungi.

2.7 Anti-microbial assay

2.7.1 By Agar-well Diffusion Assay: The anti-microbial effects of the three extracts of *C. decidua* stem were assayed on several microbial strains by the agar-well diffusion method.

2.7.1.1 Principle

The anti-microbial constituents present in the plant extracts were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zone of inhibition was uniformly circular as there was a confluent lawn of growth. The diameter of zone of inhibition was measured in millimeters.

2.7.1.2 Procedure

- MHA and SDA media plates were prepared by pouring 20 ml MHA and SDA molten media into sterile petri-plates and the plates were allowed to solidify for 5 minutes.

- The plates were seeded with the bacterial and fungal strains by spreading 100 µl inoculum uniformly on the plates using sterile spreader.

- Once the plates were dried aseptically, 6 mm wells were bored using a sterile cork borer.

- 50 µl of the plant extracts were added into the wells using sterile micropipette.

- 50 µl of Ciprofloxacin and Griseofulvin were used as the standard antibiotics and DMSO was used as negative control.

- The plates were kept for the diffusion of the plant extracts for 2 h.

- Then the plates were incubated for 24 h at 37°C for bacterial strains and 72 h at room temperature for fungi.

- The antibacterial and antifungal activities were assayed by measuring the diameter of the clear zones of inhibition formed around the well using the antibiotic zone reader.

2.8 Determination of Minimum Inhibitory Concentration (MIC)

2.8.1 By Broth Microdilution Susceptibility Test

The MIC values were also determined for the microorganisms, which were found to be sensitive to the extracts in agar-well diffusion assay, by microdilution method.

2.8.2 Procedure

• Serial dilutions of the extract, which gave the best inhibition in the agar-well diffusion test, were made and 50 µl of the diluted extracts were added in test tubes containing Mueller Hinton Broth and Sabouraud's Dextrose Broth.

• All the test tubes were inoculated with 100 µl of a calibrated suspension i.e., 108 cfu/ml the microorganism to be tested and incubated at 37°C for 18 h.

• One control test tube was prepared without the extract and it served as positive growth control.

• Ciprofloxacin was used standard antibacterial agent, whereas Griseofulvin as antifungal agent.

• At the end of incubation period, the tubes were visually examined for turbidity and cloudiness. Turbidity indicates that bacterial growth has not been inhibited by the concentration of the extract in the medium. So, the highest dilution showing at least 99% inhibition zone (without turbidity) was considered as MIC.

In primary screening, 5000 µg/ml, 2500 µg/ml, 1000 mg/ml and 500 µg/ml concentrations of the extracts were taken. Then based on the results of primary screening, the extracts were further tested in a second set of dilution against all microorganisms.

3. RESULTS AND DISCUSSION

3.1 Macroscopical Study: *C. decidua* is an immensely branched, glabrous, shrub or a small tree, varying in height from 1.5 to 5 m. Figure 1 represented the macroscopy of *C. decidua.*

Figure 1: Macroscopy of *C. decidua*

3.1.1 Root

Roots were cylindrical, tortuous, highly spreading and deep, tapering at the distal end, 1 to 6 cm in diameter; thin wiry rootlets; rough surface, longitudinally grooved and ridged, often exfoliated at places; fibrous fracture; creamish brown externally and internally white with indistinct odour and taste.

3.1.2 Stem

It was highly branched; slender, cylindrical, and glabrous, up to 5 m in height and varying in diameter from 0.1 to 2.5 cm and slightly bent at each node, giving a zig-zag appearance. Internodes were 1.5 to 4 cm long and fracture was fibrous; slightly swollen nodes, 0.5 to 2.5 cm in diameter with a pair of small stipular thorns, which were hard, woody, straight, sharp and pale brown colored, 4 to 6 mm long and 1 to 2 mm in diameter. Young stems were generally dark green in color, but they become pale green to brownish when dried and as the plant grows old, the stems developed a whitish-gray colored bark. The stems had a characteristic odour and taste.

3.1.3 Leaves

Leaves were present only on young shoots, so mature branches were leafless because of the caducous nature of leaves. Leaves were very small, about 4 to 10 mm long and 1 to 2 mm wide,

sessile or with very short petiole, simple, opposite, thick, green in color, linear-oblong with acute to mucronate apex, entire margin, glabrous surface, reticulate venation and characteristic odour and taste.

3.1.4 Fruits

Fruits were small globular or ovoid, fleshy berry, 0.5 to 2 cm in diameter, slightly beaked at the apex, with glabrous and glossy surface and about 1 cm long pedicel. They were green when fresh, reddish-pink when ripe and blackish when dried and have a characteristic odour and taste.

3.1.5 Seeds

Each fruit contains numerous seeds, embedded in the pulp. Seeds were globular, small, 1 to 3 mm in diameter, with a smooth, mucilaginous creamish-brown colored, hard and woody testa. They have indistinct odour and mucilaginous taste.

3.2 Microscopical Study

Freehand transverse sections (T.S.) of young and old stems of fresh samples of *C. decidua* were taken and studied for their histological characters.

3.2.1 Transverse section of young stem

The transverse section of young stem was presented in figure 2. The transverse section (T.S.) of young stem is circular in outline and revealed the presence of following:

3.2.1.1 Epidermis: It consists of a single layer of tangentially running cells, covered with thick and papillose cuticle. It showed the presence of sunken stomata at frequent intervals. Trichomes were absent.

3.2.1.2 Hypodermis: It followed the epidermis and was made up of 2 to 5 layers of elongated, compactly arranged palisade-like cells containing chloroplasts. These cells were alternated at intervals by a group of 5 to 20 elongated and lignified sclereids.

3.2.1.3 Cortex: It consisted of 5 to 15 layers of isodiametric parenchymatous cells. In the peripheral part of cortex, 2 to 3 cells wide band of thick-walled, lignified and pitted stone cells were seen. Cortex also showed a group of 4 to 50 small-sized, thick-walled and lignified cortical fibres and sometimes few scattered stone cells also.

3.2.1.4 Pericycle: Pericycle was present over the phloem and consisted of few lignified pericyclic fibres and stone cells also.

3.2.1.5 Stele: It consisted of narrow zone of phloem, composed of sieve tissues, companion cells and parenchyma, which encircled a wide xylem, composed of radially arranged or scattered xylem vessels, tracheids, fibres and parenchyma. The medullary rays were uni to triseriate and pitted, extending up to and becoming wider in the phloem region.

3.2.1.6 Pith: A small Pith was present in the centremost part which consisted of pitted and lignified cells.

3.2.2 Cell content: Numerous irregular-shaped prisms of calcium oxalate, prisms with plugs and simple or compound starch grains were found throughout the parenchymatous tissues in the transverse section, especially in the medullary rays.

3.2.3 Transverse section of old stem

It showed a similar structure, but epidermis and hypodermis were replaced by cork, which was made up of 10 to 20 layers of tangentially elongated and radially arranged lignified cells. A band of lignified stone cells was seen below epidermis, followed by 5 to 8 layers of cortex. Cortical fibres and pericyclic fibres were very few. Stele region also showed secondary phloem, ceratenchyma and secondary xylem. Pith was wide and well-developed.

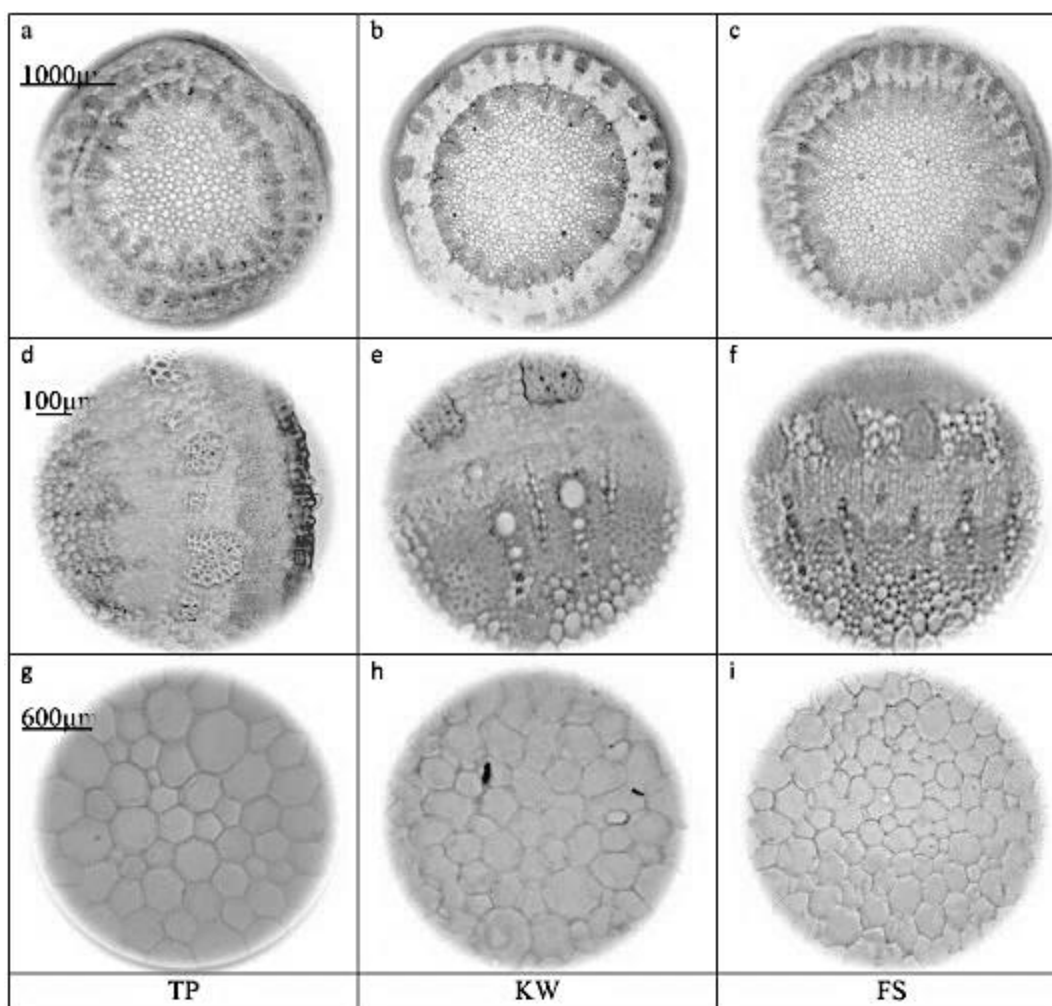

Figure 2: Detailed T.S. of young stem of *C. decidua*

3.3 Determination of Physico-chemical Parameters

The powder of *C. decidua* stem was studied for loss on drying, ash values and extractive values (Table 1).

Table 1. Physico-chemical parameters of *C. decidua* stem

Quality Parameters	Values (%w/w ± S.D.)
Loss on drying	43.21 ± 0.203
Ash value	
Total ash	7.13 ± 0.030
Acid insoluble ash	0.60 ± 0.019
Water soluble ash	5.93 ± 0.025

Extractive value	
Water soluble extractive	20.00 ± 0.135
Alcohol Soluble extractive	6.75 ± 0.127

Standard deviation (± S.D.); Number of readings (N) = 3

3.4 Screening of Anti-microbial activity

3.4.1 Primary screening for anti-microbial activity

The primary anti-microbial screening of all the three extracts of *C. decidua* stem (Extracts - A, B and C, viz. aqueous, alcoholic and 50% hydroalcoholic extracts, respectively) was carried out on Gram positive bacteria like *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, Gram negative strains like *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Erwinia carotovora* and fungal strains like *Candida albicans* and *Aspergillus niger*. The results of the anti-microbial screening of *C. decidua* stem extracts are shown in Table 2 and Figure 3.

Table 2: Evaluation of anti-microbial effects of *C. decidua* stem extracts by agar-well diffusion method

Name of Pathogens	**Diameter of Zone of Inhibition (in mm)**				
	DMSO	**STD**	**EXT-A**	**EXT-B**	**EXT-C**
Bacillus subtilis	0	17	10	13	11
Staphylococcus aureus	0	24	12	19	13
Staphylococcus epidermidis	0	21	14	20	15
Enterococcus faecalis	0	20	12	18	19
Escherichia coli	0	21	15	16	14
Klebsiella pneumoniae	0	19	17	21	17
Pseudomonas aeruginosa	0	25	19	24	20
Proteus vulgaris	0	24	15	25	18
Erwinia carotovora	0	16	12	22	16
Candida albicans	0	23	22	25	23
Aspergillus niger	0	20	0	0	0

DMSO - Dimethyl sulfoxide (Negative control); STD - Standard antibiotic (Ciprofloxacin or Griseofulvin, 5 mg/well); Ext-A - Extract-A (5 mg/well); Ext-B - Extract-B (5 mg/well) and Ext-C - Extract-C (5 mg/well).

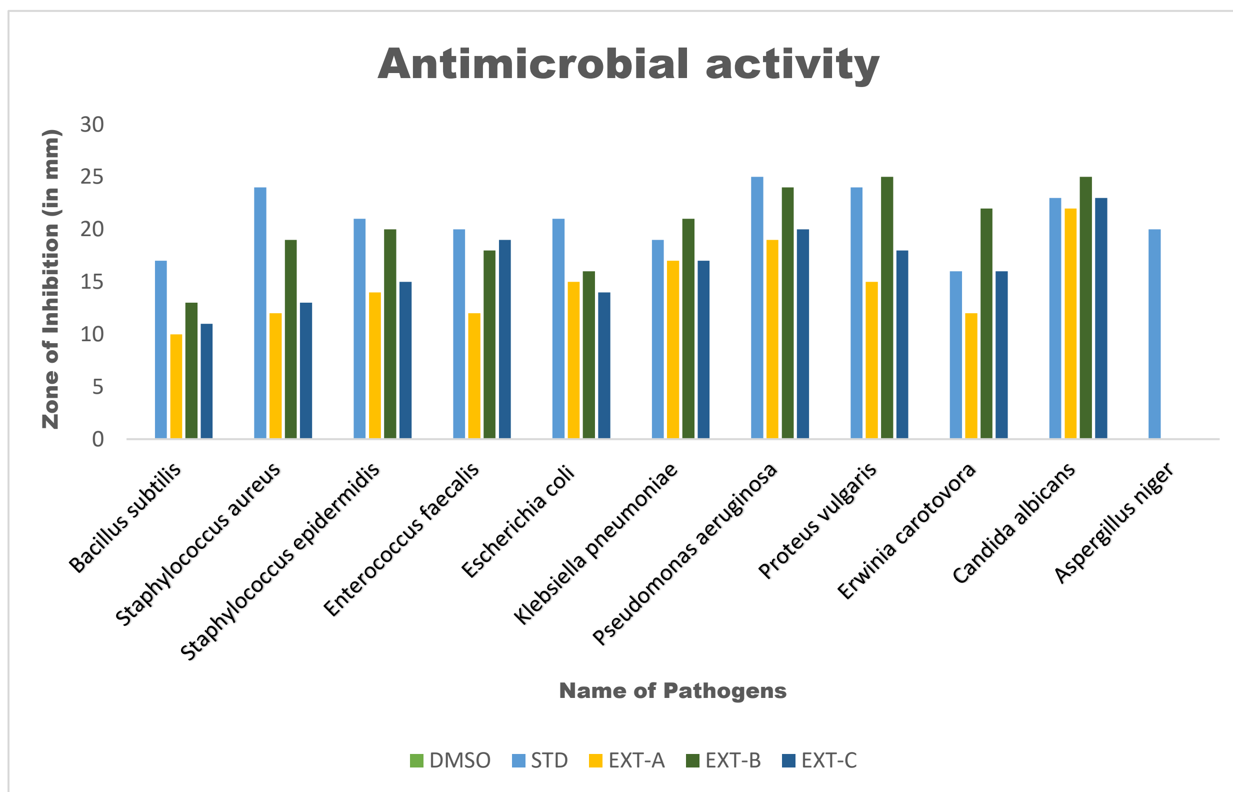

Figure 3: Antimicrobial activity of *C. decidua* stem extracts

DMSO - Dimethyl sulfoxide (Negative control); Cipro - Standard drug, Ciprofloxacin; Ext-A - Extract-A; Ext-B - Extract-B; Ext-C - Extract-C; Bs - *B. subtilis*; Sa - *S. aureus*; Se - *S. epidermidis*; Ef - *E. faecalis*; Ec - *E. coli*; Kp - *K. pneumoniae*; Pa - *P. aeruginosa*; Pv - *P. vulgaris* and Ec - *E. carotovora*

As evident from the diameters of the zone of inhibition, all three extracts of *C. decidua* stem (at a concentration of 5 mg/well) showed antibacterial activity inhibiting all the bacterial strains tested. Though all three extracts showed promising antibacterial activity, Extract-B (alcoholic extract) showed the highest activity than Extract-A (aqueous extract) and Extract-C (50% hydroalcoholic extract) and its effects were comparable to the standard antibiotic, Ciprofloxacin. However, it was observed that the tested extracts were more active against Gram-negative than Gram-positive bacteria. In the case of the Gram-negative bacteria, *Pseudomonas aeruginosa*, Extract-B gave

inhibition zones very close to the standard drug. In the case of *Klebsiella pneumoniae* and *Erwinia carotovora*, the inhibition was even greater than the standard drug.

It was also observed that all the extracts possess significant antifungal activity against *C. albicans*, but the extracts did not inhibit *Aspergillus niger*. The fungal strain *C. albicans* was inhibited strongly by Extract-B with the diameter of the inhibition zone similar to the standard antibiotic, Griseofulvin.

3.4.2 Determination of MIC

Based on the results of agar-well diffusion assay, only alcoholic extract (Extract-B) was used in the determination of MIC values against all the bacterial strains and only one fungal strain, C. albicans (Table 3, Figure 4). MIC values of Extract-B were between 50 to 500 µg/ml as observed, while those of standard antibiotics were between 250 to 500 µg/ml. The MIC value of Extract-B against Klebsiella pneumoniae (150 µg/ml) was lower than Ciprofloxacin (200 µg/ml), suggested that the extract was more effective. Moreover, the MIC values of Extract-B against *Pseudomonas aeruginosa* and *Erwinia carotovora* were found to be similar to Ciprofloxacin, and that against *Candida albicans* was found to be similar to Griseofulvin, indicated the similar effectiveness of the Extract-B as that of the standard drugs.

Table 3: MIC values of Extract-B of *C. decidua* stem for sensitive bacterial and fungal strains

Name of Pathogens	**Minimum Inhibitory Concentrations (µg/ml)**	
	Standard antibiotic (STD)	**Extract- B**
Bacillus subtilis	225	500
Staphylococcus aureus	50	225
Staphylococcus epidermidis	150	250
Enterococcus faecalis	200	150
Escherichia coli	125	150
Klebsiella pneumoniae	250	125
Pseudomonas aeruginosa	50	50
Proteus vulgaris	100	150
Erwinia carotovora	250	250
Candida albicans	125	125

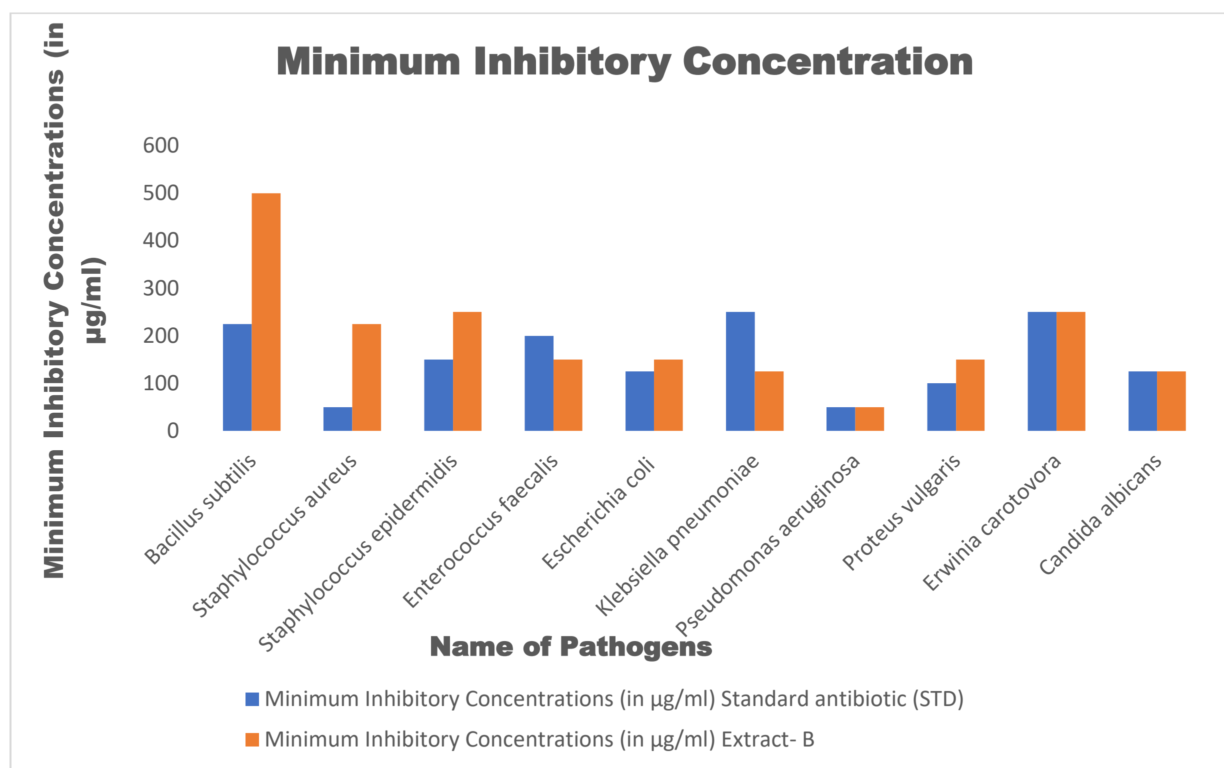

Figure 4: MIC values of Extract-B of *C. decidua* stem for sensitive bacterial and fungal strains STD - Standard antibiotic; Ext-B - Extract-B; Bs- *Bacillus subtilis*; Sa - *S. aureus*; Se - *S. epidermidis*; Ef - *E. faecalis*; Ec - *E. coli*; Kp - *K. pneumoniae*; Pa - *P. aeruginosa*; Pv - *P. vulgaris*; Ec - *E. carotovora*; Ca - *C. albicans*.

Based on the results, it is possible to conclude that *C. decidua* stem exhibited a broad range of anti-microbial activity to varying degrees. Particulary, alcoholic extract of the stem (Extract-B) showed profound antibacterial and anti-candidal activities and could be used as anti-microbial agents in new drug therapy. The study also provided support to the traditional claims of the effectiveness of this plant in various types of infections. However, the anti-microbial study can be further extended to evaluate the potential of the plant on various other pathogenic organisms and to determine and isolate the bioactive compounds responsible for the anti-microbial effects of *C. decidua* plant.

4. Conclusion

In conclusion, the study demonstrated the broad-spectrum antimicrobial potential of *C. decidua* stem extracts, with the alcoholic extract (Extract-B) showing the highest efficacy against both bacterial and fungal strains. Extract-B exhibited significant activity, particularly against Gram-negative bacteria such as Klebsiella pneumonia, Pseudomonas aeruginosa, and Erwinia

carotovora, with inhibition zones comparable to or exceeding the standard antibiotic Ciprofloxacin. Additionally, Extract-B displayed potent antifungal activity against Candida albicans, with MIC values matching the standard antifungal drug, Griseofulvin. These findings validated the traditional medicinal use of *C. decidua* in treating infections and suggest its potential as a source of natural antimicrobial agents for drug development. However, further studies are needed to isolate and characterize the bioactive compounds responsible for these effects, evaluate their mechanisms of action, and explore the plant's efficacy against a broader range of pathogens. This research supports the integration of *C. decidua* into modern therapeutic applications while emphasizing the need for additional pharmacological and toxicological investigations.

5. Conflict of interest

The authors have no conflict of interest.

6. Acknowledgement

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