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Anti-microbial potential of *Capparis decidua* stem extract against pathogenic bacteria

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Abstract
The rise of antimicrobial resistance necessitates the search for novel therapeutic agents. This study investigated the antibacterial potential of *Capparis decidua* stem extracts against multidrug-resistant bacterial strains. Ethanolic extract (EECD) and its derived fractions—chloroform-soluble (CSF), acetone-soluble (ASF), and acetone-insoluble (AIF)—were evaluated using agar well diffusion and broth dilution methods.
Results demonstrated that both EECD and CSF exhibited significant antimicrobial activity. The CSF fraction showed superior efficacy, particularly against *Escherichia coli*, with a maximum zone of inhibition (ZOI) of 26.28 mm and a minimum inhibitory concentration (MIC) of 204.6 µg mL^{-1}. The EECD was most effective against *E. coli* (ZOI: 22.42 mm; MIC: 242.3 µg mL^{-1}), followed by *Bacillus subtilis*. In contrast, the ASF and AIF fractions displayed markedly lower activity. The ethanolic extract showed the least inhibition against *Pseudomonas aeruginosa* (ZOI: 14.65 mm).
The findings confirm that *Capparis decidua* stem possesses potent antibacterial compounds, especially within its chloroform-soluble fraction. This validates its traditional medicinal use and highlights its promise as a source for developing complementary antimicrobial agents against drug-resistant pathogens.

Keywords: Antimicrobial resistance, *Capparis decidua*, antibacterial activity, ethanolic extract

Introduction
Antimicrobial resistance, which is probably the result of the selection pressure of antimicrobial usage, has made several types of antimicrobial medications less effective. The use of herbs for medicinal purposes is getting more and more popular, which could be connected to the fact that several frequent and less costly conventional antimicrobial medicines are becoming less effective because germs are developing resistant to numerous medications [1, 2]. Numerous plants with important medicinal uses are native to India. To be investigated for usage as pharmaceuticals and the treatment of common illnesses, the plant's pharmacological qualities—such as its antibacterial, analgesic, antipyretic, and antioxidant properties—must also be known [3-5].
Creating novel antibacterial drugs has become essential in the fight against the rise of infections that are resistant in recent times. Providing safe and effective complementary and alternative medicine treatments may prove to be a crucial tool in improving access to healthcare, since over one-third of people in developing countries lack access to essential pharmaceuticals (WHO 2002) [6, 7]. The prior literature research makes it abundantly evident that these plants' acute toxicity investigations and antibacterial properties (both *in vitro* & *in vivo*) against MDR bacterial strains have not been investigated for potential therapeutic uses. Furthermore, the different phytochemical components' antimicrobial qualities were not investigated. Although some of the therapeutic properties of *Capparis decidua* have been reported in other investigations, more extensive research is still needed [8-10].
No research has been done on the antibacterial properties of *Capparis decidua* extract or how it affects strains of this plant that are resistant to many drugs. As such, it would appear important to investigate the phytochemical makeup and important medicinal properties of *Capparis decidua* stem [11].
The plant *Capparis decidua* has therapeutic uses. It has been shown to have antimicrobial potency against a variety of microbes [12]. The antibacterial properties of *Capparis decidua* are believed to be attributed to its biologically active constituents, which include swerchirin, opelic acid, amarogentin, xanthones, triterpenoids, and gentiopicric [13]. It can aid with a wide range of ailments, including as diarrhea, bronchial asthma, anemia, fever, stomach disorders, and liver disorders. It also has a wide range of biological characteristics, such as antihelminthic, antibacterial, anticancer, hepatoprotective & hypotensive effects [14]. The anti-microbial qualities of the ethanol extracts from *Capparis decidua* are examined in this study.

Material & Methods
1. Collection of Plant
Capparis decidua Stem bark will be obtained from the local area & selected drugs will be authenticated by the expert taxonomist.

2. Preparation of Stem bark Extract: Extraction Methodology
The dried stem bark will be coarsely powdered and extracted with ether by a Soxhlet apparatus at 80°C. The marc was dried and again extracted in Soxhlet extractor using ethanol as a solvent. Respective extracts were prepared by vacuum drying and concentrate them [15].

3. Phytochemical Study
A small amount of freshly prepared plant methanolic crude extract was subjected to a preliminary qualitative phytochemical analysis. Using the standard procedure that follows, phytochemicals such as polyphenolic, flavonoids,

glycosides, alkaloids, steroids, saponins, carbohydrates, and proteins were identified [16].

4. Comparative Physico-Chemical Analysis

In the current study, the physicochemical properties of powdered plant samples were evaluated. The methodology employed to ascertain these parameters was executed in compliance with the directives [17].

Antibacterial Assay for antibiotics

Antibacterial screening via agar well diffusion method

The anti-microbial effects of the three extracts of C. decidua stem were assayed on several microbial strains by agar-well diffusion method.

Principle

The anti-microbial constituents present in the plant extracts are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zone of inhibition is uniformly circular as there is a confluent lawn of growth. The diameter of zone of inhibition is measured in millimetres [18].

Result & Discussion

Collection, Procurement and Authentication of plant material

The fresh young and mature stems of *Capparis decidua* were collected from local area, Alwar, during flowering season. The authenticity was established by comparing its morphological characters with the available literature and was further confirmed by a botanist at Department of Botany, RR College, Alwar. Herbarium of the collected sample was prepared and a voucher specimen no. NIPR/Pharmacognosy/2024/01 was deposited in Department.

Physico-chemical parameters

Result and discussion

Drug moisture content should be minimized to prevent the growth of bacteria, yeast, or fungi while being stored. Extractive value is helpful for estimating the chemical constituents that are soluble in the specific extraction solvent and for evaluating crude drugs since it provides insight into the types of chemical constituents contained in the medication.

Ash values are crucial quantitative benchmarks and evaluation criteria for determining the authenticity and purity of raw materials, particularly when they're in powder form. Furthermore, a crude drug's total ash content indicates the level of care used in both the preparation and storage of the drug. A component of total ash called acid insoluble ash quantifies the amount of silica, particularly in the form of sand and siliceous earth. The amount of total ash that is soluble in water is known as water soluble ash.

Table 1: Extractive values and color of extract in different solvent

Extract	Color of Extract	Extractive value (% w/w)
Petroleum ether (60-80°C)	Pale green	1.95
Hexane	Green	1.45
Acetone	Greenish Brown	3.26
Ethanol	Greenish Brown	8.2%
Aqueous/Water	Brownish green	5.45%

Table 2: Proximate Analysis of *Capparis decidua* crude drug

Physicochemical Parameter	Value in % w/w for Stem Extract
Total Ash Content	7.45± 0.36 %
Water-soluble ash value	4.25 ± 0.15 %
Acid-insoluble ash value	0.72± 0.08 %
Sulphated-ash value	1.25 ± 0.04 %
Moisture content	8.12 %
Volatile oil	1.2 %
pH	5.6
Foreign matter	1.2 %

The extractive value of crude powder was maximum in ethanol (8.2%), followed by water (5.45%) and minimum was in hexane (1.95%). pH and melting point of ethanol extract was 5.6 and 123°C respectively.

Ethanolic extraction of *Capparis decidua* stem

Percentage yield of chloroform soluble fraction and acetone insoluble fractions were calculated. The ethanol extract and different fraction were stored for pharmacological screening. Percentage yield, consistency and color of different fraction of *C. decidua* stem are shown in table 3.

Table 3: % yield, consistency and color of different extracts / fraction of *C. decidua* Stem

S. no.	Extraction /fraction	Colour	Consistency	(% w/w) Yield
1	Petroleum ether extract	Light brown	Sticky mass	1.9%
2	Ethanol extract	Dark brown	Sticky mass	8.1%
3	Chloroform soluble extract	Dark brown	Sticky mass	36.12 % of ethanol extract
4	Acetone soluble fraction	Dark brown	Sticky mass	25.8 % of ethanol extract
5	Acetone insoluble fraction	Dark brown	Sticky mass	38.1 % of ethanol extract

Results and discussion

The percentage yield of petroleum ether and ethanol extract of *C. decidua* Stem was found to be 1.9 %, 8.1 %, where as chloroform soluble extract, acetone soluble fraction and acetone insoluble fraction of ethanolic extract were found *36.12* %, 25.8%, and 38.1% respectively. These extracts and fractions were stored in airtight container for further studies.

Qualitative Phytochemical Assay

Plant extracts from *Capparis decidua* have been shown to have elements having medicinal promise. Alkaloids, flavonoids, amino acids, phenolics, steroids, tannins, and saponins are found in the ethanolic extract of the *Capparis decidua* plant and may be involved in several pharmacological activities.

Table 4: Qualitative chemical tests of extracts of *Capparis decidua*

Phyto-constituents	Tests	Present (++) or Absent (--)
Alkaloids	▪ Dragendorff's Test ▪ Mayer Test	++ +
Flavonoids	▪ Shinoda test ▪ Fluorescence test	++ ++
Phenolics	▪ Test with $FeCl_3$ ▪ Test with Folin ciocalteu reagent	++ ++
Tannins	▪ Test with gelatin ▪ Test with lead acetate	++ +
Saponins	▪ Froth test ▪ Haemolytic zone test	+ +
Sterols and triterpenoids	▪ Libermann Burchardt's test ▪ Salkowski reaction	– –
Carbohydrates	▪ Molisch's test ▪ Fehling test	+ –
Coumarins	▪ Test with ammonia ▪ Test with hydroxylamine hydrochloride	– –
Anthraquinone glycosides	▪ Borntrager's test ▪ Modified Borntrager's test	– –
Cardenolides	▪ Baljet test ▪ Keller killiani's test	– –

Antibacterial activity of Ethanol extracts & Fractions of stem of *C. decidua*

Result & Discussion

The growth of all bacterial strain was significantly affected by the application of plant extract and fractions. The examined plant's stem ethanol extract showed inhibition towards the test organisms. For gram-positive organisms, the ZOI for S. aureus was 18.39 mm at the least and 19.2 mm at the highest for B. subtilis, and range was 14–20 mm. regarding gram-negative bacteria, P. aeruginosa displayed 14.65 mm at maximum intensity while E. coli showed 22.42 ZOI in conc 50-µl/ml.

It was found that ethanolic extract of the stem of *C. decidua* effectively inhibited the growth of all selected bacteria but its maximum inhibiting potential was recorded against *E. coli* with greater inhibition zones (22.42 mm) and lower MIC values (242.3 µg mL^{-1}) followed by *B. Subtilis* (19.2 mm; MIC, 252.7 µg mL^{-1}), while Chloroform soluble fraction of ethanol extracts produce more inhibition compared to pure extract with highest zones (26.28 mm; MIC, 204.6 µg mL^{-1}) for *E. coli* and 21.74 MIC, 270.2.6 µg mL^{-1} for *S. aureus* as given in Table 6.8 and 6.9. In case of C. decidua stem bark, ethanolic extract show minimum inhibiton of *P. aeruginosa (*14.65 mm) and in case of CSF it was *B. Subtilis* with (14.65 mm) inhibition.

With a zone of inhibition (ZOI) extending from 10–19 mm for *Bacillus species* & 11–18.5 mm for *P. aeruginosa*, the ethanol extract of the stem (EECD) demonstrated less activite against both of these bacteria. The ASF and AIF of ethanol extract from the stem shows less inhibition of all microorganisms. For Acetone soluble fraction *E. Coli* showing the highest zone of inhibition 15.44mm. Bacillus species, K. pneumoniae, and P. aeruginosa all demonstrated a negligible zone of inhibition. Similarly, Acetone insoluble fraction (AIF) shows highest zone of inhibition (16.25mm) for *P. aeruginosa* and lowest for *K. pneumonia* (7.45mm ZOI).

Table 5: Antimicrobial activity (mm) of stem extract and their fractions of *C. decidua* (assessed by disc diffusion assay)

Plant Extract	Treat ment	Zone of inhibition (mm)				
	(50 µL)	***S. aureus***	***P. aeruginosa***	***E. coli***	***K. pneumoniae***	***B.* Subtilis**
EECD	Ethanol extract	18.36	14.65	22.42	15.54	19.2
	Chloroform soluble fraction	21.84	17.46	26.28	18.2	16.45
	Acetone soluble fraction	12.36	11.65	15.44	10.54	11.24
	Acetone insoluble fraction	16.25	14.82	9.45	7.45	10.28
Standard	Ciprofloxacin (5mcg)	27.8	29.36	31.7	25.45	28.24
	Amoxicillin (30mcg)	30.82	31.5	34.25	28.88	30.16

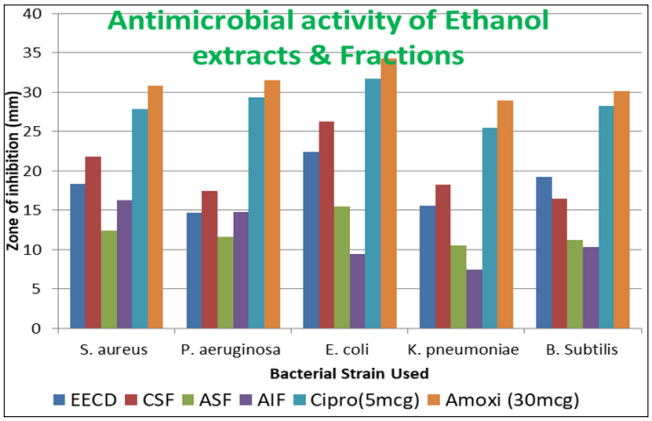

Fig 1: Antibacterial activity against a range of bacterial species is demonstrated by the ethanol extract and fractions of *Capparis decidua*

Antibacterial activity assay by minimum inhibitory concentrations (MIC)

The effectiveness of the extracts in tested bacterial strains was determined by measuring the minimum inhibitory concentration (MIC). MIC was performed for only those organisms which showed a zone of inhibition and were sensitive to the plant extracts in the previous antimicrobial assay by agar well diffusion method. Among all plant extract and fractions tested, EECD and CSF were found to show strong antimicrobial activity. The minimum inhibitory concentrations of Ethanol extract and chloroform fractions of *Capparis decidua* was assessed by standard sensitive serial dilution microplate method against the all bacterial strains in triplicates. This assay was selected for the reason that of its simplicity, sensitivity, reproducibility, and low cost along with the fast procedure.

Table 6: MIC value of plant extracts against microorganisms (mg/mL)

Cultures	Gram	MIC (µg /ml)		
		Ethanol Extract	Chloroform Soluble Fraction	Ciprofloxacin (5mcg)
S. aureus	Gram-+VE	282.4±5.1	270.2±3.6	35.5±1.2
B. Subtilis	Gram-+VE	252.7±2.8	236.2±2.6	24.3±0.6
P. aeruginosa	Gram--VE	330.5±4.2	295±4.6	32.6±0.8
E. coli	Gram--VE	242.3±5.1	204.6±5	36.4±0.8
K. pneumoniae	Gram--VE	470.4±7.5	455.7±7	52.1±1.6

Conclusion

The research work results indicate inhibition against certain gram -ve as well as gram +ve bacteria. The interpretation of the antibacterial sensitivity results of the investigated leaves & stem ethanol and methanol extracts against the five different bacterial strains. With a ZOI extending from 14–19 mm for Bacillus species & 10–18 mm for P. aeruginosa, the ethanol extract demonstrated considerable activity against both of these bacteria. At lower doses, S. aureus, E. coli, and K. pneumoniae showed resistance, & a zone of inhibition was only noticeable at higher concentrations. The leaves exhibited a remarkable action against Bacillus species and P. aeruginosa, E. coli, and K. pneumoniae. In every instance, S. aureus showed total resistance to the extract concentrations [19, 20].

In conclusion, it was discovered that the alcoholic extracts from the medicinal plants (stem) exhibited antibacterial activity against all of the microorganisms used in the current study and had significant antimicrobial activity against five strains of microorganisms. These extracts would be an interesting source for the discovery of new antibiotics derived from plants [21].

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