

IN VITRO ANTIBACTERIAL ACTIVITIES OF DIFFERENT SOLVENT EXTRACTS OF CAJANUS CAJAN L. SEED COAT AND COTYLEDONPRATIMA H^{1*}, PRATIMA MATHAD²¹Department of Postgraduate Studies and Research in Botany, Akkamahadevi Women's University, Vijayapur, Karnataka, India.²Department of Postgraduate Studies and Research in Botany, Gulbarga University, Kalaburgi, Karnataka, India.

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ABSTRACT

Objectives: The present study was conducted to assess the antibacterial activity of seed coat and cotyledon of *Cajanus cajan* with various solvents such as petroleum ether, chloroform, ethanol, and aqueous.

Methods: The crude extracts were obtained using Soxhlet successive extraction method. The antibacterial activity of seed coat and cotyledon extracts at different concentration (50 and 100 mg/mL) was evaluated against certain pathogenic Gram-positive bacteria of *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* and Gram-negative bacteria of *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* by agar well diffusion assay.

Results: The results show that the ethanolic extract of seed coat had highest activity against *B. subtilis* (22.0±0.13 mm). Whereas, the aqueous extract of cotyledon had highest activity against *S. aureus* (20.0±0.02 mm) and *S. typhi* (20.0±0.04 mm) at 100 mg/mL. The *S. pneumoniae* and *P. aeruginosa* were completely resistant to all extracts of seed coat. Similarly, *S. pneumoniae*, *K. pneumoniae*, *E. coli*, and *P. aeruginosa* were totally resistant to all tested cotyledon extracts. The phytochemical results show the presence of alkaloids, steroids, phenols, flavonoids, tannins, lignins, glycosides, and absence of saponins in seed coat and cotyledon.

Conclusion: This study shows that *C. cajan* seed coat and cotyledon had potential antibacterial activity against *B. subtilis*, *S. aureus*, and *S. typhi*. These extracts may be exploited for the development of antimicrobial and alternative remedies for infections and diseases caused by respective pathogens.

Keywords: Antibacterial activity, *Cajanus cajan*, Cotyledon, Phytochemicals, Seed coat.

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INTRODUCTION

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The secondary metabolites of plants such as alkaloids, flavonoids, tannins, and phenolic compounds are considered as valuable phytochemicals found to be used as an intermediate for the production of new drugs [1]. The study of antibacterial activity is more important in the current years due to the development of antimicrobial drug resistance and often the occurrence of undesirable side effects of some antibiotics [2]. Plants are safer alternative sources of antimicrobials [3,4].

The most commonly consumed legume in India is *Cajanus cajan* (L.) Millsp (Pigeonpea) and is also the fifth most important pulse in the world. The *C. cajan* being a forage crop has been utilized as a remedy for various ailments. Studies have proved that the *C. cajan* seeds, when added to coffee alleviates headache and vertigo [5]. There are reports on isolation of isoflavones, namely, daidzein (4, 7-dihydroxy-isoflavone) and formononetin (7-hydroxy- 40-methoxy isoflavone) from the seed germs [6]. The seed extracts of *C. cajan* exhibit hypolipidemic, antioxidant, and antimicrobial activities [7,8]. The seed coat of leguminous plant constitutes major by-product of milling industries. It is utilized mainly as cattle feed and also in manufacture of biscuits [9], and cotyledon used as "dal" in our daily diet. The seed coat has been proved to be an excellent material for treating wastewaters containing low concentration of metal ions [10]. Seed coats act as a potential great resource for the development of antioxidant and antihyperglycemic nutraceuticals. [11]. Antibacterial activity has already been reported in leaf, seed, root, pod, and flower extracts of *C. cajan* [12-14]. Literature survey revealed that the seed coat and cotyledon extracts have yet not been screened for its traditional claim of antibacterial activity and little

attention was paid on medicinal properties of seed coat and cotyledon. Hence, the present work was carried out to explore the antibacterial properties of seed coat and cotyledon of *C. cajan*.

METHODS**Plant material collection**

The seeds of *C. cajan* var. Maruti (ICP-8863) belonging to the family Fabaceae were collected from Agriculture Research Station, Kalaburgi, Karnataka state, India. The variety Maruti ICP-8863 (PN-ABR-333) was authenticated by plant material description no. 44, International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India [15].

Processing of sample

The seeds were moistened for 1 h and then dried in oven at 55°C overnight. The hull or seed coat was removed mechanically using hand grinder. The two fractions such as seed coat and cotyledon were made into fine powder using grinder.

Preparation of extract

500 g of the powdered seed coat and cotyledon were successively extracted by the Soxhlet extractor method (60-80°C) using 2.5 L of petroleum ether (PE), chloroform (CHCl₃), ethanol (Et-OH 95%, v/v), and water (Aq) solvents in the increasing order of polarity for 18 h. The extracts obtained were dried at 40°C.

Phytochemical screening

The seed coat and cotyledon extracts were screened for alkaloids, flavonoids, glycosides, saponins, tannins, phenols, lignins, and steroids as described by Harborne [16].

Test microorganisms

The bacterial strains used in the present study are *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* a Gram-positive and *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* a Gram-negative organism, which were obtained from the Department of Microbiology, Gulbarga University, Kalaburgi, Karnataka, India. They were maintained at 4°C on the slants of nutrient agar medium for further use.

Reconstitution of the extracts

1 g of petroleum ether, chloroform, and ethanol and aqueous extracts were reconstituted with 2 mL of dimethyl sulfoxide to obtain a concentration of 50 and 100 mg/mL of each extract.

Screening of antibacterial activity

The agar well-diffusion method was used to determine the inhibitory effects of the extracts against the microbes [17]. The bacterial isolates obtained were first grown in nutrient broth for 18 h at 37°C. Simultaneously, Mueller–Hinton Agar medium was prepared and 18 mL of medium was poured into sterile Petri dishes while hot and then they are allowed to set till solidified. After this stipulated period, 0.2 mL of the broth culture of the bacteria was aseptically inoculated on this plate using a sterile cotton swab and allowed to dry. A standard sterile cork borer of 8 mm diameter was used to cut four uniform wells on the surface of the agar. Four wells on each isolate plate were filled with each of the crude extracts with the aid of a sterile syringe. In the similar way, one agar plates for each microorganism were prepared for studying the activity of standard streptomycin (0.4 mg/mL). The plates were incubated at 37°C for 18–24 h and observed for zones of inhibition. A zone of clearance around each well signifies the inhibition, and the diameter of such zones was measured in millimeter (mm) with a transparent ruler [18].

Statistical analysis

The data of all measurements are means from three replications. Data and statistical significance of difference were evaluated with analysis of variance using SPSS 10.0 package.

RESULTS

Phytochemicals

The phytochemicals detected in *C. cajan* seed coat and cotyledon extracts are listed in Table 1. Test for flavonoids, tannins, phenols, glycosides, alkaloids, steroids, and lignins were positive in all extracts. However, alkaloids and steroids are not detected in the PE and chloroform extracts of seed coat and cotyledon, whereas the saponins was absent in all extracts of seed coat and cotyledon.

Antibacterial activity

Antibacterial activity of different solvent extracts of seed coat of *C. cajan* was assayed against various Gram-positive and Gram-negative bacterial pathogens by agar well-diffusion method as shown in Table 2. The ethanolic extracts of seed coat showed the highest activity on both Gram-positive and Gram-negative bacteria when compared with

the aqueous, chloroform, and PE extracts, except *S. pneumoniae* and *P. aeruginosa*. However, the PE and chloroform extract were resistant to *S. aureus*, and PE extract was resistant to *S. typhi*. The ethanolic extract of seed coat was more sensitive against *B. subtilis* (22.0±0.13 mm) followed by *S. aureus* (18.0±0.02 mm), *K. pneumoniae* (18.0±0.09 mm), *S. typhi* (15.0±0.08 mm), and *E. coli* (14.5±0.06 mm) at 100 mg/mL, respectively.

Different solvent extracts of cotyledon showed significant antibacterial activity against *B. subtilis*, *S. aureus*, and *S. typhi*. Whereas, *S. pneumoniae*, *K. pneumoniae*, *E. coli*, and *P. aeruginosa* were totally resistant to all tested extracts of cotyledon (Table 3). The aqueous cotyledon extract was the most effective against the *S. aureus* and *S. typhi* at 100 mg/mL. The *B. subtilis* shows maximum inhibition zone in ethanolic extract (15±0.12 mm) followed by PE extract (10.0±0.02 mm) at 100 mg/mL. The chloroform and aqueous extracts were resistant to *B. subtilis*. Similarly, *S. aureus* showed maximum inhibition zone in the aqueous extract (21.0±0.02 mm) followed by ethanol (16.0±0.04 mm) and PE (13.0±0.01 mm) at 100 mg/mL. Chloroform extract was resistant to *S. aureus*. The *S. typhi* showed maximum inhibition zone in the aqueous extract (20.0±0.04 mm) than for the ethanol extract (15.0±0.03 mm) at 100 mg/mL, the PE and chloroform extracts were resistant to *S. typhi*.

DISCUSSION

The plant extracts proved promising natural antimicrobial agents with potential applications in infectious disease for controlling pathogenic bacteria. The phytochemical tests revealed the presence of flavonoids, tannins, phenols, glycosides, alkaloids, steroids, and lignins in seed coat and cotyledon of *C. cajan*. Biologically, important specific compounds have been identified in leaf extracts of the *C. cajan* [19,20]. The demand for more natural antimicrobial has driven scientists to examine the effectiveness of inhibitory compounds such as extracts from plants [21]. Secondary metabolites such as tannins, steroid, alkaloids, flavonoids, and glycosides have the antimicrobial properties [22,23]. The presence of these metabolites may support the antibacterial activity of seed coat and cotyledon extracts of *C. cajan*.

The present results show that seed coat extract was more sensitive against Gram-positive and Gram-negative bacteria compared to cotyledon extracts. The method of extraction and the microorganism to be targeted would be important factors to consider for optimal activity of the extract. The growth inhibitory effect usually depends on concentration [24]. This is important to identify the dosage and rate at which the extract inhibits the growth of organism [25]. The study revealed that higher concentration at 100mg/ml of ethanolic extract of seed coat shows maximum activity against *B. subtilis* and aqueous extract of cotyledon was more sensitive against *S. aureus* and *S. typhi*. The difference in the observed activities of the various extracts may be due to varying degrees of solubility of the active constituents in the solvents used. It has been documented that different solvents have diverse solubility capacities for different phytoconstituents [26]. Doppalapudi has reported the better action

Table 1: Preliminary phytochemical screening of seed coat and cotyledon of *C. cajan* L.

Secondary metabolites	Seed coat				Cotyledon			
	PE	CHCl ₃	Et-OH	Aq	PE	CHCl ₃	Et-OH	Aq
Phenols	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Lignins	+	+	+	+	+	+	+	+
Sterols	-	-	+	+	-	-	+	+
Alkaloids	-	-	+	+	-	-	+	+
Glycosides	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-

+: Present, -: Absent, PE: Petroleum ether, CHCl₃: Chloroform, Et-OH: Ethanol, Aq: Aqueous extracts. *C. cajan*: *Cajanus cajan*

Table 2: Antibacterial activity of seed coat extracts of *C. cajan*

Extracts microorganisms	Zone of inhibition in mm					
	Concentration mg/mL	PE	Chloroform	Ethanol	Aqueous	Streptomycin 0.4 mg/mL
Gram-positive bacteria	50	12.0±0.2	13.0±0.05	16.0±0.05	12.0±0.05	25.0±0.1
<i>B. subtilis</i>	100	13.0±0.4	13.5±0.15	22.0±0.13	12.0±0.02	
<i>S. aureus</i>	50	-	-	13.0±0.04	14.0±0.07	28.0±0.0
	100	-	-	18.0±0.02	15.0±0.03	
<i>S. pneumoniae</i>	50	-	-	-	-	19.0±0.5
	100	-	-	-	-	
Gram-negative bacteria	50	-	12.0±0.05	10.0±0.05	14.0±0.04	21.0±0.2
<i>S. typhi</i>	100	-	13.0±0.02	15.0±0.08	12.0±0.5	
<i>K. pneumoniae</i>	50	11.0±0.02	12.5±0.06	13.0±0.2	12.0±0.12	22.0±0.5
	100	13.0±0.05	14.0±0.04	18.0±0.09	13.0±0.05	
<i>E. coli</i>	50	11.0±0.04	12.0±0.02	14.0±0.04	13.0±0.02	15.0±0.5
	100	13.0±0.05	14.0±0.04	14.5±0.06	14.0±0.04	
<i>P. aeruginosa</i>	50	-	-	-	-	27.0±0.2
	100	-	-	-	-	

Each value is expressed as mean±SD. (n=3) and statistically significant at P<0.05. *B. subtilis*: *Bacillus subtilis*, *S. aureus*: *Staphylococcus aureus*, *S. pneumoniae*: *Streptococcus pneumoniae*, *S. typhi*: *Salmonella typhi*, *K. pneumoniae*: *Klebsiella pneumoniae*, *E. coli*: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*

Table 3: Antibacterial activity of cotyledon extracts of *C. cajan*

Extracts microorganisms	Zone of inhibition in mm					
	Concentration mg/mL	PE	Chloroform	Ethanol	Aqueous	Streptomycin 0.4 mg/mL
Gram-positive bacteria	50	09.0±0.13	-	12.0±0.01	-	25.0±0.1
<i>B. subtilis</i>	100	10.0±0.02	-	15±0.12	-	
<i>S. aureus</i>	50	12.0±0.05	-	13.0±0.04	15.0±0.05	28.0±0.0
	100	13.0±0.01	-	16.0±0.04	21.0±0.02	
<i>S. pneumoniae</i>	50	-	-	-	-	19.0±0.5
	100	-	-	-	-	
Gram-negative bacteria	50	-	-	12.0±0.07	15.0±0.02	21.0±0.2
<i>S. typhi</i>	100	-	-	15.0±0.03	20.0±0.04	
<i>K. pneumoniae</i>	50	-	-	-	-	22.0±0.5
	100	-	-	-	-	
<i>E. coli</i>	50	-	-	-	-	15.0±0.5
	100	-	-	-	-	
<i>P. aeruginosa</i>	50	-	-	-	-	27.0±0.2
	100	-	-	-	-	

Each value is expressed as mean±SD. (n=3) and statistically significant P<0.05. *B. subtilis*: *Bacillus subtilis*, *S. aureus*: *Staphylococcus aureus*, *S. pneumoniae*: *Streptococcus pneumoniae*, *S. typhi*: *Salmonella typhi*, *K. pneumoniae*: *Klebsiella pneumoniae*, *E. coli*: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*

of aqueous and ethanolic extracts of seeds of *Vigna unguiculata* against *B. subtilis* and *E. coli* [27]. There are reports on the ethanolic and aqueous extracts of *C. cajan* leaves, and seeds were effective in producing inhibition zones against *S. aureus*, *E. coli*, and *P. aeruginosa* [14]. Similarly, Banala *et al.* have reported the ethanolic and methanolic extract of leaf, root, pod, and flower extracts of *C. cajan* against *S. aureus* and *E. coli* [12].

CONCLUSION

This study shows that ethanolic and aqueous extract of seed coat and cotyledon of *C. cajan* had potential antibacterial activity against Gram-positive and Gram-negative bacteria, and these extracts may be exploited for the development of anti-microbial and alternative remedies for infections and diseases caused by respective pathogens. Further studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest

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