Medicinal potential of antimicrobial peptides from two plants against *Bacillus cereus* and *Staphylococcus aureus*

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ABSTRACT

Bacillus cereus and *Staphylococcus aureus* are the most important bacteria that cause nosocomial infection and are resistant to antibiotics. Crude proteins from *Cassia fistula* and *Ricinus communis* were isolated to study their medicinal potential against *Bacillus cereus,* and *Staphylococcus aureus.* Extraction of the crude proteins from plants was done by phosphate buffer saline (PBS) and Tris NaCl buffer by using the roots and seeds of both plants. Antimicrobial activity was checked against bacterial strains by using agar disc diffusion and agar well diffusion methods. Zones of inhibitions were measured. On well diffusion method, PBS buffer protein extract of *C. fistula* roots showed a maximum zone of inhibition of 25 mm against *B. cereus*. Tris NaCl buffer extracts of *C. fistula* roots and seeds showed zones of inhibition of 12mm and 5mm respectively against *S. aureus* while *Ricinus communis* roots showed a zone of 12mm against *B. cereus*. Because the protein of the plants showed good antimicrobial activity, we can use these plants against various diseases caused by *Bacillus cereus* and *Staphylococcus aureus.*

Introduction

The collection of wild medicinal plants is a tradition that has been practiced in many rustic populations in Pakistan.[1] Because it gets larger in the number of contaminations and the harmful effects of available antibiotics, ancient medicinal plants are being admired on chemical drugs.[2] Furthermore, the appearance and gradual rise of antibiotic resistance in microorganisms increase the demand to find other sources of treatment from plants.[3] Most of the plants are available as potential medicinal drugs and can serve as a supply for effective new chemicals with antimicrobial activity. Almost all of the plants form peptides with antimicrobial activity (antimicrobial peptides, AMP) for example seeds contain many active constituents that have remarkable enzymatic, fungicidal activity, and non-enzymatic proteins.[4] Plants synthesize several proteins to guard themselves against pathogen assaults.[5] These are host defensive peptides that have their role in innate immune response, they also induce and potentiate several other plants defending procedure that leads to pathogen death.[6] These proteins perform their antimicrobial (medicinal) function by hydrolyzing the cell wall components of microbes or other pathogens.[7] Different parts of plants hold these proteins such as leaves and seeds. Once syn-

thesized and switched on, these proteins powerfully hinder fungi, bacteria, and insect herbivores.[8] Most of these proteins specifically target bacteria while some possess a broad range of antimicrobial activity through different mechanisms.[9] The medicinal potential of AMP is documented in many studies against many micro-organisms which prove that these proteins are concerned with host plant defense against microbial attack.[10] Several plant AMPs have been reported and filtered from many plants that show a remarkable range of significant biological potencies about defense. These potencies include ion-channel blocking and antimicrobial potency.[11] Numerous researches display the medicinal activity of protein extracts of important medicinal plants.[12]

Ricinus communis (castor oil plant) is a flowering plant of the family Euphorbiaceae.[13] Its roots and leaves have medicinal properties, and it is used mainly for rheumatism, inflammations, and nervous disorders. The juice of leaves is given for dysentery. Many lesser metabolites are produced in plants which act as a source of microbicides, pesticides, and pharmaceutical drugs.[14]

Ricinus communis also contains different compounds which show antimicrobial properties. These compounds including their leaf, root, and seed oil significantly possess remedial potential towards inflammation and liver disorders.[15] It is effective against gram-positive as well as gram-negative bacteria.[16] *Cassia fistula* is another flowering plant good to the family Fabaceae that is known for its medicinal activity.[17] The plant contains different compounds which show antimicrobial properties. It includes tannins, flavonoids, and glycosides.[18] *Cassia fistula* species act as fungicides because they contain chrysophanic acid-9-anthrone.[19] Cassia species de-alcoholized seed extract inhibit the growth of *Micrococcus pyogenes var. albus, Micrococcus citreus, Salmonella schottmuelleri,* and *Escherichia coli.*[20] *Cassia fistula* is used against various diseases as a broad-spectrum antimicrobial agent.[21] Their roots and leaves have medicinal potential against Bacillus cereus and *Staphylococcus aureus*. These bacteria cause stomach and intestinal infection that leads to diarrhea and other diseases.[22]

Currently, the use of AMPs is going to be considered for the purpose of producing novel environment-friendly microbicides.[23] Since pathogenic resistance is rising gradually against antibiotics, hence decreasing antibiotics efficacy. Here we need to locate natural antibacterial methods to crack this problem.[24] This experimental study was aimed at extracting therapeutically important phytonutrients specifically the antimicrobial proteins from the two therapeutically important plants *Cassia fistula* and *Ricinis communis* belonging to the family Euphorbiaceae and Fabaceae respectively. Several AMPs have been reported to be present in these families so we picked out the plants of these two families for the extraction of proteins to carry out antimicrobial assay.

Materials and Methods

Plant sample collection

Seeds and roots of the plants were collected from the Botanical field of Cholistan Institute of Desert Studies Islamia University of Bahawalpur. Samples were authenticated and identified by expert botanist Prof. Dr. Ghazala H. Rizwani in the Department of Pharmacognosy of the University of Karachi under the herbarium voucher number A-143 of *Ricinus communis* and A-135 of *Cassia fistula*. All the samples were washed and dried (Figure 1).

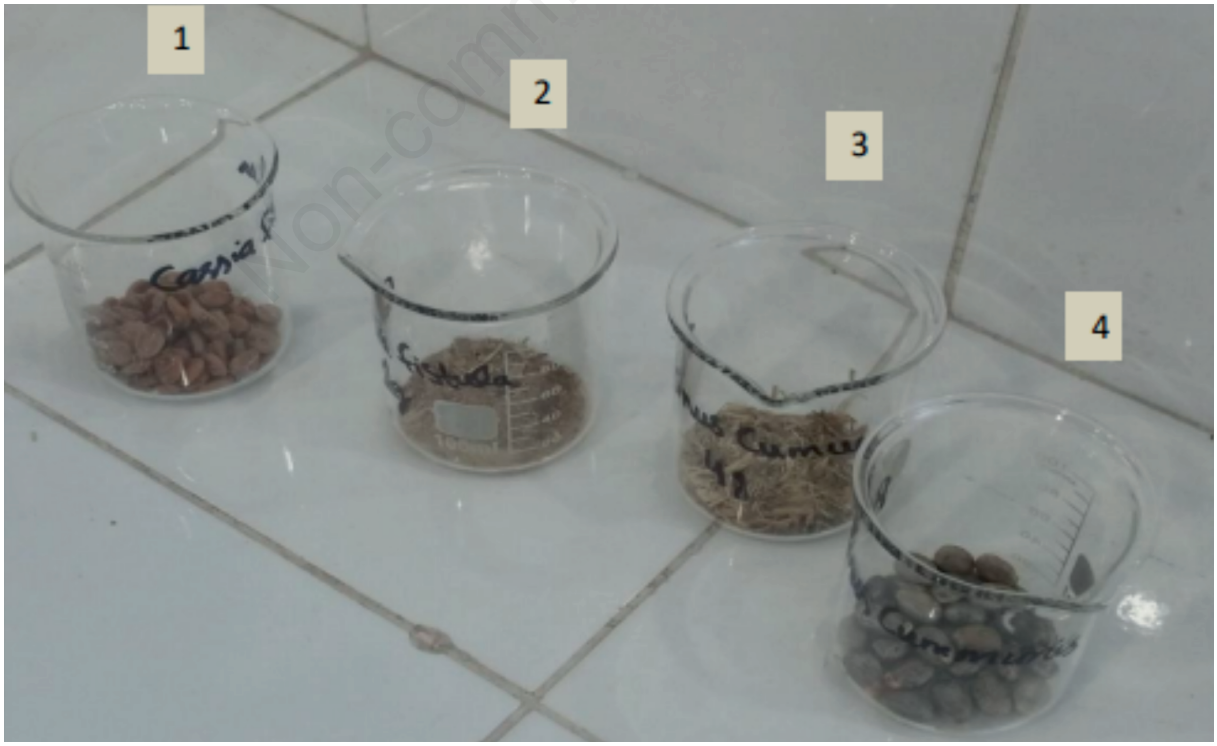

Figure 1. Plant samples 1 and 4 show seeds of *Cassia fistula* and *Ricinus communis* respectively while 2 and 3 contain roots of *Cassia fistula* and *Ricinus communis* in ground form respectively.

Antibacterial peptide extraction by Tris NaCl buffer

The pulling out of AMPs was done by milling fresh 1gm roots and 1gm seeds of each plant individually in 3.3 ml of 1 Molar Tris-HCl (pH 7.5) and 0.5 Molar NaCl. The resulting mixture of buffers was subjected to incubation for about 12 hours at 4°C. Samples were then centrifuged at 12,000rpm for 20 min. After that, the resulting supernatant was observed for further studies.

Antibacterial peptide extraction by phosphate buffer saline

The latest 0.3gm roots and seeds of each sample were powdered by grinding in the pre-chilled mortar and pestle in 4.5 ml of phosphate buffer saline (PBS). Specimens were then subjected to freeze-thaw cycles thrice at a gap of 12 hours between the cycles. Tubes were then centrifuged at 10,000 rpm for nearly 10 minutes. The resulting supernatant was collected and kept at 4°C for quantification.

Protein concentration by spectrophotometer

The total protein content of seeds and roots of plant withdrawal was examined spectrophotometrically at 595 nm. Bradford method was carried out by forming a bovine serum albumin stock solution in a mass of (2 mg/ml) for PBS and (1 mg/ml) for extracts of Tris NaCl samples. Bradford reagent was produced and thinned out 1:4 times by merging 3 ml of the reagent with 12 ml of refined autoclaved water. The reagent was then purified by Whatman #1 paper instantly before use. Bradford reagent was produced according to the recipe given.[25]

Test bacterial organisms

The bacterial organisms to be tested were provided by the Culture Collections of the Department of Microbiology and Molecular Genetics, The Women University Multan. The bacterial organisms used in the work were *Bacillus cereus*, and *Staphylococcus aureus*.

Antibacterial activity

The test organisms were grown on the Luria-Bertani Agar Media. These were covered for a whole night at 37°C. Antibacterial activity of the protein withdrawal of plants was checked by agar disc diffusion and agar well diffusion method. Zones of inhibition were examined. Chloramphenicol commercially prepared discs in a concentration of 10 ug/disc were used as positive control.

Disc-diffusion method

Discs of size 6 mm of Watman filter paper 1 were prepared. 20 ul measured quantity of plant extracts were filled on individual discs and positioned on the plates. Plates were subjected to incubation for 24 hours at 37°C in an incubator and the antimicrobial potency was calculated by measuring the zones of inhibition in millimeters by graduated scale.[26]

Well diffusion method

Wells of approximately 5 mm in diameter were created in plates containing the solidified agar medium using, Pasteur pipette at an equal distance apart from each other. The measured 50 ul of each plant protein extract was dropped in the corresponding well. Plates were then kept at room temperature for half an hour and then incubated at 37 degrees for 24-48 hours. After that, the zone of inhibition was calculated in millimeters with the help of a graduated scale.[27]

Results

Concentration of plant protein sample in spectrophotometer

The transmission density of protein extracts of each plant root and seed sample was premeditated on the spectrophotometer at 595 nm. The gathering of plant protein samples withdrawn through different buffers is given in Table 1. The highest protein concentration of 2,353 ug/ml was established in Cassia fistula root withdrawn by PBS buffer with freeze-thaw cycles, and without freeze-thaw cycles the concentration was 2,228 ug/ml while the protein cluster of the same plant with Tris NaCl buffer was 562.66 ug/ml.

According to the table results, the protein mass in plant extracts in PBS buffer with freeze-thaw was more than the extracts in Tris NaCl buffer. The mass of protein was higher in PBS buffer with freeze-thaw cycles, as protein mass in Ricinus communis roots with PBS buffer with freeze-thaw cycles was 718 ug/ml while in the without-freeze-thaw cycles it was 0.056 ug/ml. The concentration of the same plant from Tris NaCl buffer was 199.33 mg/ml, which was lower than the PBS buffer extrication protein concentration.

Table 1. Protein concentration of samples.

Plants	Tris buffer		PBS buffer with freez- thaw		PBS buffer without freeze-thaw	
	O.D	µg/ml	O.D	µg/ml	O.D	µg/ml
Cassia fistula (roots)	0.187	562.66	0.451	2353	0.426	2228
Cassia fistula (seeds)	0.249	739.33	0.291	1553	0.200	1098
Ricinus communis (roots)	0.078	199.33	0.124	718	0.037	0.056
Ricinus communis (seeds)	0.290	90.6	0.274	1468	0.301	1603

PBS, phosphate buffer saline.

Disc diffusion method

Antibacterial activity of protein extracts through Tris NaCl buffer

A zone of inhibition of contrasting bacterial strains was fixed by using the protein withdrawal of plants and Chloramphenicol. None of the protein withdraw showed any antibacterial activity against any strain (Figure 2).

Well diffusion method

Antibacterial activity of protein extracts through Tris NaCl buffer

Purified protein samples were examined for antibacterial activity by using a well diffusion method. Zone of inhibition of 12mm and 5mm was shown against *Staphylococcus aureus* by roots and seeds of *Cassia fistula* respectively (Figure 3)

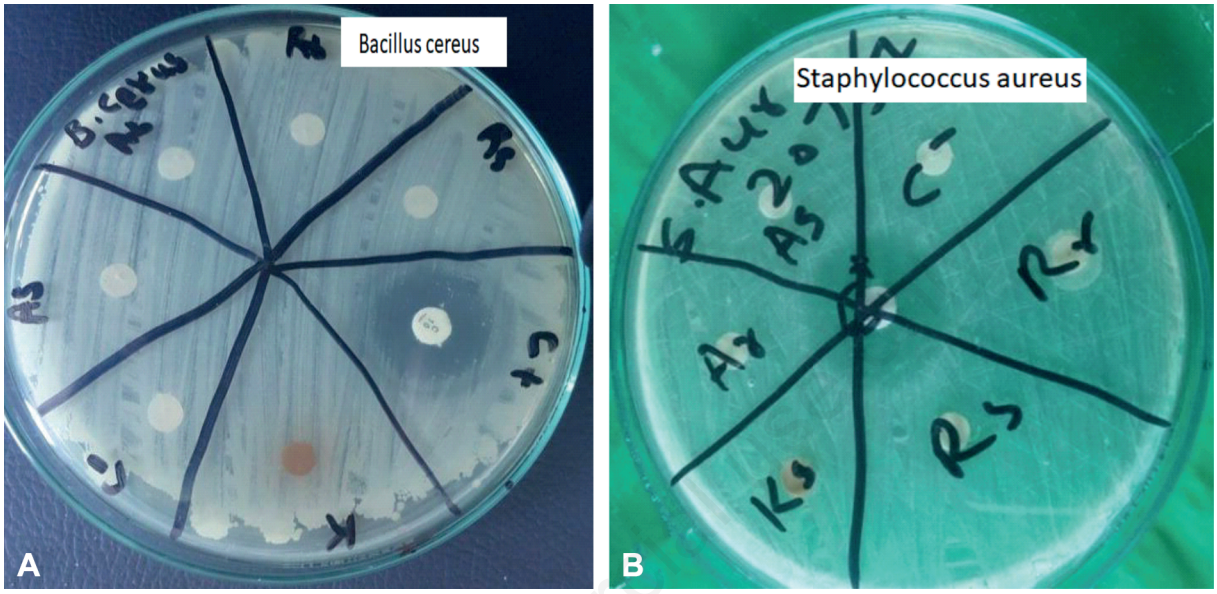

Figure 2. Zone of inhibition of crude protein extracts through Tris NaCl buffer A) *Bacillus cereus* B) *Staphylococcus aureus*. Protein extraction of *Cassia fistula* seeds (As), *Cassia fistula* roots (Ar), *Ricinus communis* seeds (Rs), *Ricinus communnis* roots (Rr).

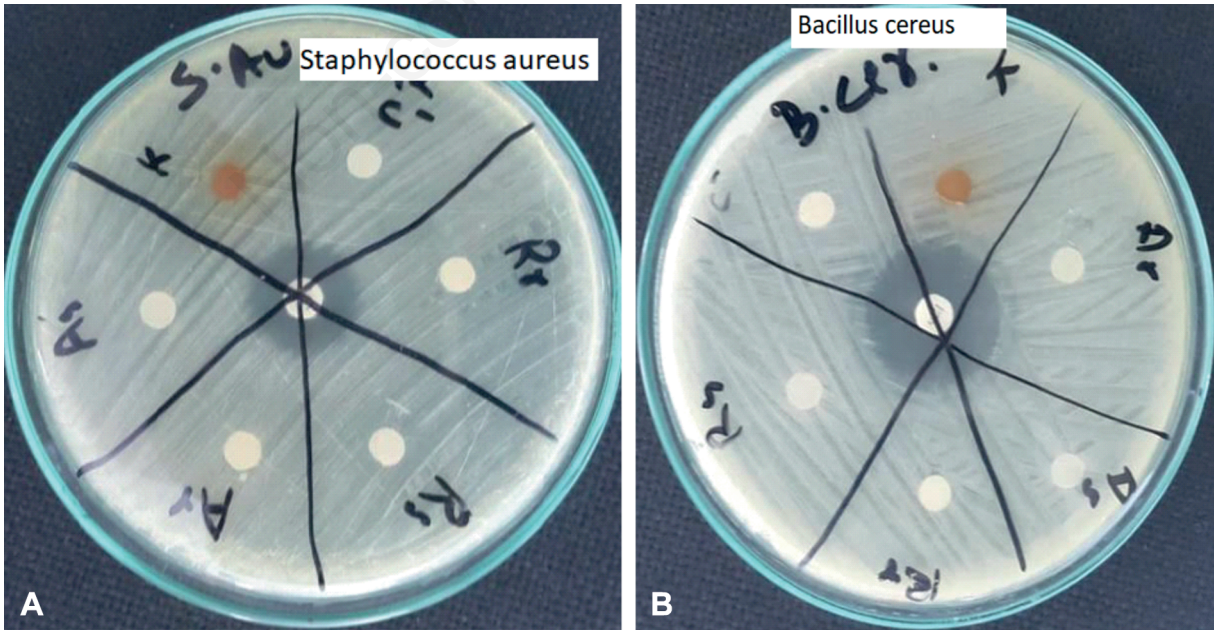

Figure 3. Zone of inhibition of crude protein extracts through phosphate buffer saline buffer A) *Staphylococcus aureus* B) *Bacillus cereus*. Protein extraction of *Cassia fistula* seeds (As), *Cassia fistula* roots (Ar), *Ricinus communis* seeds (Rs), *Ricinus communnis* roots (Rr).

and zone of 15mm appeared against *Bacillus cereus* by *Ricinus communis* roots (Table 2).

Antibacterial activity of protein extracts through phosphate buffer saline buffer

Antibacterial assay was done using well diffusion method against *Bacillus cereus* and *Staphylococcus aureus.* Cassia fistula root extract shows antimicrobial activity against *staphylococcus aureus* with a zone of inhibition of 5mm and *Bacillus cereus* with 15mm (Figure 4).

Discussion and Conclusions

Pathogenic bacteria are examined as a chief source of fatality particularly in evolving countries.[28] Herbs are fiercely used in conventional medicine and their therapeutic potentials are well registered.[29] Our study was to check the medicinal activity of *Cassia fistula* seeds (As), *Cassia fistula* roots (Ar), R*icinus communis* seeds (Rs), and *Ricinus communis* roots (Rr) by using Tris NaCl buffer crude protein extract and PBS crude protein extract against gram-positive bacteria including *Bacillus cereus,* and *Staphylococcus aureus.* In this examination, two buffers Tris Nacl and PBS buffer were used for the protein withdrawal, and the antibacterial activity was checked by agar disc diffusion and agar well diffusion method (Tables 3 and 4). Protein concentration with only 20µl was impregnated on the disc while 50µl of crude protein extract was used in agar well diffusion method. Agar well diffusion method showed better antibacterial activity than agar disc diffusion method. Upon disc diffusion method crude extract of *Ricinus communis* seeds and roots through Tris NaCl buffer didn't show zones of inhibition. Upon well diffusion method root extract of *Ricinus communis* through Tris buffer and PBS buffer showed antibacterial activity with a zone of inhibition of 15mm and 10mm respectively against *Bacillus cereus.* Al-Mamun reported the antibacterial activity of *Ricinus communis* seed through Tris NaCl buffer against pathogen by using concentrations of 50, 100, 200, and 400 µl/disc.[30] All bacteria showed the best results at 50µl/disc. Verma also demonstrated that the root extract of *Ricinus communis* shows antimicrobial properties against various pathogenic microorganisms such as *Escherichia coli and Staphylococcus aureus* by well diffusion method.[31] Al-Mamun said that Aqueous extract doesn't show considerable antimicrobial effects. The hexane and methanol extracts showed the highest antimicrobial potency. Antibacterial activity of *Ricinus communis*is considered to be due to the lectin protein's action such as ricin in the castor bean.[32] *Ricinus communis* contains heterodimeric protein, which possesses cytotoxic activity by interrupting protein synthesis and therefore can be applied to kill cancer cells.[33]

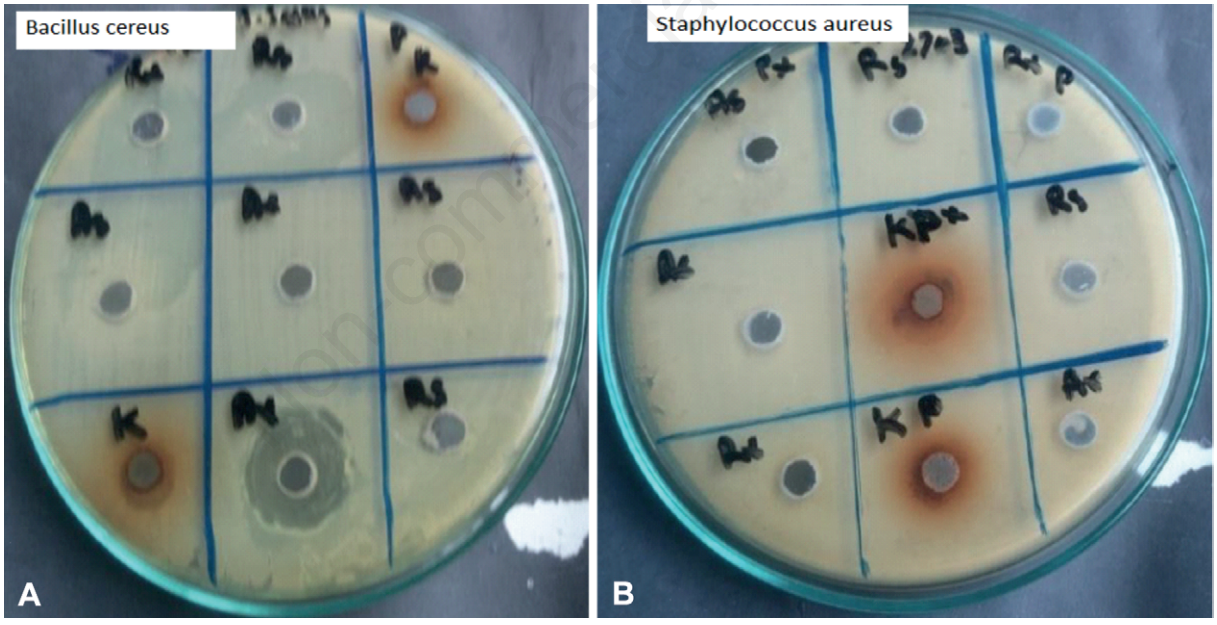

Figure 4. Zone of inhibition of crude protein extracts through Tris NaCl buffer A) *Bacillus cereus* B) *Staphylococcus aureus*. Protein extraction of *Cassia fistula* seeds (As), *Cassia fistula* roots (Ar), *Ricinus communis* seeds (Rs), *Ricinus communnis* roots (K).

Table 2. Zone of inhibition of phosphate buffer saline protein extract through disc diffusion method.

Bacteria	Plant sample				
	C. Fistula roots	*C. Fistula* seeds	*R. communis* roots	*R. communis* seeds	Control
S. aureus	---	---	---	---	15 mm
B. cereus	---	---	---	---	20 mm

Table 3. Zone of inhibition of Tris NaCl protein extract through agar well diffusion method.

Bacteria	Plant sample			
	C. Fistula roots	*C. Fistula* seeds	*R. communis* roots	*R. communis* seeds
S. aureus	12mm	5mm	---	---
B. cereus	---	---	15mm	---

Table 4. Zone of inhibition of phosphate buffer saline protein extract through agar well diffusion method.

Bacteria	Plant sample			
	C. Fistula roots	*C. Fistula* seeds	*R. communis* roots	*R. communis* seeds
S. aureus	---	---	---	---
B. cereus	25mm	---	10mm	---

In this study, *Cassia fistula* root and seed extract through Tris NaCl buffer showed no activity at 20µl/disc. The PBS buffer protein extract of *Cassia fistula* roots and seeds showed antimicrobial activity of 5mm each on disc diffusion method. Through well diffusion method using Tris NaCl buffer *Cassia fistula* roots and seeds showed zones of 12mm and 5mm respectively against *S. aureus*. The antibacterial potency of *Cassia fistula* chloroform seed withdrawal was examined against two gram-positive *S. aureus*, *S. pyogens*, and two gram-negative bacteria named *P. aeruginosa*, and *E. coli* .[34] Yadava reported that by using agar disc diffusion method and determine the zone of inhibition. Crude extract showed antibacterial activity against various strains. Zone of inhibition compared with different standards Ciprofloxacin, Chloromphenicol, and Norfloxacin.[35] Isolated compounds from *Cassia fistula* root extract showed antimicrobial and antitubercular properties against the bacteria *Staphylococcus aureus* and *Bacillus subtilis.* Channabasappa isolated the compound from *Cassia fistula* which showed antimicrobial potency against *Staphylococcus aureus, Bacillus subtilis*, and *Fusariumoxysporum*.[36] *Cassia fistula* root withdrawal also showed antibacterial potency against various microorganisms as well as gram-positive and gram-negative bacteria. Its activity was checked against 5 gram-positive and 9 gram-negative bacteria which included *Sarcinalutea*, *Bacillus megaterium*, *shigella flexneri*, *shigellashiga*, and *Klebsiella pneumoniae*. The extract showed no activity at 30µg/disc but showed good activity at 200µg/disc. The size of the zone of inhibition (9-14mm) indicates their low sensitivity.[37] Jabeen also reported extraction of *Ricinus communis* seed protein through PBS *Ricinus communis*. Seed extract through PBS buffer showed maximum activity than Tris Buffer. All extracts showed maximum activity with PBS. So, buffers play an important role in its activity.[38] Similarly well diffusion method showed maximum activity than disc diffusion method because in disc diffusion method the concentration of protein is 50µl but in disc diffusion, it is only 20µl.[37] The extract showed no activity at 30µg/disc but showed good activity at 200µg/disc. There is also a chance of disc contamination in disc diffusion method.

Habiba in her research conducted the extraction of antimicrobial peptides from the leaves of 4 important medicinal plants, results showed the antimicrobial activity of *Albizia lebbeck* PBS protein extract by maximum zone of inhibition of 17 mm against *Bacillus cereus*.[39]

The present study concluded that PBS buffer protein withdrawal of *C. fistula* roots showed a higher zone of inhibition of 25 mm against *Bacillus cereus* while Tris NaCl buffer extracts of *Ricinus communis* roots showed a zone of 12mm against *B. cereus* on well diffusion method. Hence, AMP extracted from *C. fistula* and *R. communis* roots have the maximum antibacterial activity against bacterial strains and protein extracts of these plants can be used for the synthesis of antimicrobial drugs to treat bacterial infections.

An increasing number of bacterial infections cause potential health problems and existing antibacterial drugs have numerous side effects so the preparation of antibacterial drugs from protein extracts of plant sources can serve as the best alternative to treat these infectious diseases.[40]

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