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Research Article

Antimicrobial Effects of Ethanolic Extracts from *Cuscuta reflexa* Roxb. (Convolvulaceae)

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ABSTRACT
Ethanolic whole plant extracts obtained from *Cuscuta reflexa* Roxb were screened against Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram negative (*Escherichia coli* and *Salmonella typhi*) bacteria to evaluate their antimicrobial activity. Of the four concentrations of plant extract tested (200 µg/mL, 300 µg/mL, 400 µg/mL or 500 µg/mL), 500 µg/mL elicited the greatest zones of bacterial inhibition across three of the bacteria. In contrast, the growth of *Salmonella typhi* was not halted regardless of extract concentration. At 200 µg/mL, only the growth of *E. coli* was inhibited. Overall, although the greatest antimicrobial activity was demonstrated to be against *E. coli* at a concentration of 500 µg/mL (24.6±0.24), upon comparison to the other bacteria, both *B. cereus* and *S. aureus* educed similar zones of inhibition upon comparison to their positive antibiotic control.

Keywords: Antimicrobial activity, *Cuscuta reflexa* Roxb, agar well diffusion, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi.*

INTRODUCTION

The history of plant usage for their medicinal qualities dates back centuries across a multitude of cultures. Extraction and characterization of several active phytocompounds from a variety of plants have given rise to highly active profile drugs[1]. Parallel with population explosions is the increased number of virulent microorganisms, hence triggering higher infection and mortality rates[2]. In Bangladesh, a country densely populated, the minimization of infectious diseases is a great challenge within the health and economic sector. In an attempt to prevent such diseases, a large number of antibiotics, almost all of which are synthetically derived, have been developed in the last few decades[3]. Currently, numerous antibiotics are now resistant to both Gram positive and Gram negative bacteria due to erroneous prescribing and poor self-treatment practices[4]. As a result, given the broad-spectrum efficacy of various medicinal plants, the development of novel antibacterial agents is vital to help overcome the increasing incidence of antibiotic resistance[5]. *Cuscuta reflexa* Roxb. a member of the Convolvulaceae family, is a leafless, twining parasitic plant which thrives throughout Bangladesh. With no underground root system, it is commonly known as akaswel (sky twinner) or amarbel (immortal twine), whilst in English, it is referred to as Dodder[6]. Various parts of the plant have been traditionally used to treat a wide range of ailments. Seeds from the plant are known to have a carminative and purgative effect, whilst the juice can be used as an anthelmintic or as a detoxifier to purify the blood[7]. Further, a fruit decoction can be imbibed to treat a cough and fever and the stem can be ingested for the alleviation of constipation, flatulence and liver complaints[8]. Given the diverse pharmacological actions of the plant, including potential antimicrobial activity[9]. We selected *C. reflexa* Roxb to evaluate its bactericidal potential when tested against both Gram positive and Gram negative bacteria.

MATERIALS AND METHODS

Plant Collection and Identification

The whole plant of fresh *C. reflexa* Roxb. was collected from the Banani Rail Gate area, Dhaka, Bangladesh. The plant was identified by the taxonomist of the Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh and a voucher specimen was deposited in the herbarium unit.

Plant Sample Preparation

The whole plant of *C. reflexa* Roxb. was washed in water, sun dried for twenty days and then ground using a mortar and pestle to a fine powder (69.7 g) and stored in an air tight container at room temperature until required.

Ethanolic Extraction

Approximately 50 g of powdered *C. reflexa* Roxb. was incubated in 300 mL of ethanol in a sealed container wrapped in aluminium foil for three days at room temperature with occasional shaking and stirring. The ethanolic extract was then filtered at room temperature through Whatman filter paper and the ensuing filtrate was stored in an air tight container until required.

Test Microorganisms

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Table 1: Antimicrobial activity of ethanolic extracts obtained from *Cuscuta reflexa* Roxb.

Bacteria	µg/mL				
	control	200	300	400	500
Bacillus subtilis	12.7±0.11	0±0.0	5.4±0.36	6.8±0.14	8.8±0.19
Staphylococcus aureus	19±0.0	0±0.0	8.8±0.12	10.9±0.04	13.7±0.0
Escherichia coli	35.9±0.12	17.9±0.04	19.9±0.08	22.9±0.08	24.6±0.24
Salmonella typhi[*]	7.0±0.0	0±0.0	0±0.0	0±0.0	0±0.0

Note: Values represent the diameter of zone of inhibition (mm). All results are presented as mean ±SEM of triplicate data sets.

[*]denotes duplicate replicates only.

The bacterial strains used for this study (*Bacillus subtilis. Staphylococcus aureus*, *Escherichia coli*, *Salmonellae typhi*) to assess antimicrobial susceptibility were kindly donated by Primeasia University, Department of Pharmacy, Dhaka, Bangladesh.

Antimicrobial assay

The agar well diffusion assay[10] was used with the following minor adjustments to evaluate antimicrobial efficacy. Briefly, nutrient agar plates were evenly inoculated with individual microorganisms using a sterile cotton swab and then wells were aseptically punched into the agar using a 6 mm biopsy punch. 50 uL of ethanolic extracts at concentrations of 200, 300, 400 and 500 µg/mL were added to each well. Commercially available antibiotic discs of amoxicillin (30 µg for *S. aureus*, *B. subtilis* and *S. typhi*) and kanamycin (30 µg for *E. coli*) served as the positive controls whilst sterile water acted as the negative control. All plates were incubated at 37°C for 24 hours and subsequent zones of inhibition were measured (millimetres). Data is represented as ±SEM of triplicate data sets unless denoted.

RESULTS

Overall, as the concentration of plant extract increased (200 µg/mL – 500 µg/mL), so did the zone of inhibition in three of the four bacteria tested thereby signalling a dose-dependent effect (Table 1). In contrast, the growth of *S. typhi* was not halted regardless of concentration. At 200 µg/mL, only *E. coli* was inhibited (17.9±0.04), whilst the concentration of 500 µg/mL elicited the greatest bactericidal effects against *S. aureus* (13.7±0.0), *B. subtilis* (8.8±0.19) and *E. coli* (24.6±0.24). Further, none of the concentrations of plant extract tested educed a greater zone of inhibition in the bacteria that were screened compared to their cognate positive antibiotic control.

DISCUSSION

C. reflexa Roxb., a parasitic plant, is used as an anthelmintic, carminative, purgative, aphrodisiac and for the treatment of constipation to name but a few[11]. The antimicrobial activity of whole plant extracts from *C. reflexa* Roxb. were evaluated against both Gram positive (*B. subtilis* and *S. aureus*) and Gram negative bacteria (*E. coli* and *S. typhi*) using kanamycin (30 µg) and amoxicillin (30 µg) as the standard antibiotic controls. In general, plant extracts at all concentrations demonstrated the highest antimicrobial activity against *E. coli* compared to the other bacteria. A concentration of 500 µg/mL elicited the greatest zone of inhibition in all bacteria except *S. typhi* which was not susceptible to any plant extracts irrespective of dose. As shown in a previous study, the ethanolic extract of *C. reflexa* Roxb. contains a myriad of compounds such as alkaloids, carbohydrates, glycosides, flavonoids, tannins, phenolic compounds and steroids[12]. The authors determined that it is the flavonoid glycosides contained within the plant that are responsible for the inherent antimicrobial activity

CONCLUSION

This preliminary investigation suggests that the ethanolic extracts from *C. reflexa* Roxb. does possess significant antimicrobial properties as only micrograms of crude plant extract were able to elicit a bactericidal effect against both Gram positive and Gram negative bacteria. Moreover, these findings support folkloric use of this species with regards to the treatment of bacterial infections. The non-prescription use of medicinal plants is cited today as an important health problem, as many are toxic to the kidney. As such, future directions will include cytotoxicity testing, the identification of bioactive compounds and subsequent elucidation of pharmacological properties and associated mechanisms.

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Competing Interests

The authors have declared that no competing interests exist.

Consent

Not applicable.

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