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ORIGINAL RESEARCH ARTICLE

Antibacterial Activity of *Trichosanthes cucumerina* Linn. Extracts

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ABSTRACT

Trichosanthes cucumerina Linn (Family: Cucurbitaceae) is a climber grown in Asian countries including Sri Lanka, India, Malay Penisula and Philippine. The present study was conducted to evaluate whether aerial parts of *T. cucumerina* can exert antibacterial activity. The antibacterial activity of a hot water extract (HWE) and a cold ethanolic extract (CEE) of *T. cucumerina* aerial parts was evaluated by (a) viable colony count and (b) disc diffusion techniques against gram (+) and gram (–) bacterial strains such as *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aeroginosa*. Results of the present study reveal that *T. cucumerina* has components that can significantly inhibit the growth of *S. aureus, S. pyogenes, E. coli* and *P. aeroginosa*. Of the two extracts tested, CEE was found to exert consistently better antibacterial activity than HWE. In conclusion, *T. cucumerina* extracts exhibited antibacterial activity against gram (+) ve bacterial strains such as *S. aureus, S. pyogenes* and gram (-) ve bacterial strains such as *E. coli* and *P. aeroginosa.*

Key Words: *Trichosanthes cucumerina, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeroginosa*

INTRODUCTION

Wound infections are common in developing countries due to poor hygienic conditions. *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeroginosa, Streptococcus pneumoniae* and *Klebsiella pneumoniae* are some important bacterial strains causing wound infections [1] . A wide range of antibiotics (e.g. erythromycin, tetracyclines, trimethoprim, sulfonamides, gentamicin, etc) are being used at present for treating wound infections[2, 3] .Bacterial resistance to antibiotics is a major therapeutic problem and the rate at which new antibiotics are being produced is slowing[4]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents, has led to the screening of several medicinal plants for their potential antibacterial activity. There are several reports in the literature regarding the antibacterial activity of plant extracts [5, 6, 7] .

Trichosanthes cucumerina Linn. (Family: Cucurbitaceae) is one of the medicinal plants that is often used in Sri Lankan traditional systems of medicine for the preparation of formulations used to treat a variety of disease conditions [8,9]. It is widely distributed in Asian countries including Sri Lanka, India, Malay Peninsula and Philippine [8]. Experimentally, *T. cucumerina* of Sri Lankan origin has been shown to have antidiabetic [10], gastroprotective [11], anti-inflammatory [12], antioxidant [13], lipid lowering [14], activities and non toxicity [15] in rodents.According to publications on therapeutic uses of *T. cucumerina*, it is useful for treatment of wounds including boils, sores, skin eruptions such as eczema and dermatitis[8]. The presence of bacteria within a wound cause infections and delay the healing [2]. However, up to date, no investigations have been

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carried out to scientifically evaluate the antibacterial potential of *T. cucumerina*. Therefore, the aim of the present investigation was to evaluate the antibacterial activity of *T. cucumerina* against some selected bacterial strains that are known to cause wound infections.

MATERIALS AND METHODS

Plant material

T. cucumerina plants were collected from Western province of Sri Lanka between the periods of August – September. The plant was identified and authenticated by the curator of National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen (TS 01) was deposited in the Industrial Technology Institute, Colombo 7, Sri Lanka.

Preparation of hot water extract (HWE)

T. cucumerina aerial parts were cut into small pieces and air dried. Plant material (60 g) was boiled in 1.9 L of distilled water (DW) and the final volume was reduced to 240 ml by gentle boiling over 4 h and freeze dried (yield 12.5% dry weight basis). A stock solution was prepared at a concentration of 1000 µg/ml from the HWE by dissolving 10 mg of the extract in 10 ml of DW and sterilized by autoclaving at 110 ^{o}C for 10 min. and stored at - 20 ^{o}C until use.

Preparation of cold Ethanolic extract (CEE)

T. cucumerina aerial parts were air dried in the shade, cut into small pieces and macerated with ethanol (500 ml) and kept for 48 h at room temperature (28 - 30 ^{o}C). The extract was filtered and evaporated to dryness under reduced pressure at 50 ^{o}C (yield 7.5% w/w dry weight basis). A stock solution was prepared at a concentration of 1000 µg/ml from the CEE by dissolving 10 mg of the extract in 10 ml of 1% Tween 80 solution and sterilized by autoclaving at 110 ^{o}C for 10 min. and stored at - 20 ^{o}C until use.

Selection of Bacterial strains

Antibacterial activity of *T. cucumerina* extracts was evaluated against the following bacterial strains obtained from the National Collection of Type Cultures (NCTC): gram positive bacterial strains, *Staphylococcus aureus* (NCTC 25923), *Streptococcus pyogenes* (NCTC 20258) and gram positive bacterial strains, *Escherichia coli* (NCTC 25922), *Pseudomonas aeroginosa* (NCTC 20620).

Preparation of growth medium for Bacterial strains

CM 55 agar (40 g), agar powder (5 g) and Mueller Hinton agar (13 g) were added to a beaker containing 1L of distilled water. The mixture was then heated while stirring until all the solid particles were dissolved completely. The final pH of the medium was adjusted to 7.4 ± 0.2 and autoclaved at 121 ^{o}C for 15 min. under a pressure of 15 psi. When the temperature of the medium was reduced to 40 ± 5 ^{o}C, human blood (70 mL) was added after separating the serum, mixed well, and poured into petri dishes (15 ml/petri dish) and allowed to set at room temperature. These blood agar plates were stored at 4 ^{o}C until use.

Evaluation of Antibacterial Activity of the HWE and the CEE

Antibacterial activity was evaluated by use of (a) viable colony count technique and (b) disc diffusion technique

Viable Colony Count Technique

This assay was carried out according to the method described by Weerasekara and co – workers [16]. One hundred micro liters (containing 5 x 10^8 colony forming units) of each bacterial (*Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aeroginosa*) suspension was added to vials containing 900 µl from the solutions of the HWE (500 µg/ml) and the CEE (500 µg/ml) respectively. The content of each vial was mixed well and kept in an incubator at 37 ^{o}C for 60 min. For the control tubes, the same bacterial suspension (5 x 10^8 colony forming units in 100 µl) and 900 µl of sterile normal saline was added, mixed well and kept in an incubator at 37 ^{o}C for 60 min. Subsequently, 100 µl from each tube was inoculated on to blood agar plates and incubated for 24 h at 37 ^{o}C. At the end of 24 h incubation, the colony count in each plate was estimated and expressed as colony forming units per milliliter (cfu/ml). Plant extracts that completely inhibited the growth of a specific bacterial strain (i.e. no colony growth) were tested further as described below.

1 in 2 dilution series (250, 125, 62.5, 31.25, 15.62, 7.81 µg/ml) of the HWE and CEE extracts were prepared from the stock solution (500 µg/ml) of each extract. Then, 900 µl of each dilution was introduced to bacterial suspension (5 x 10^8 colony forming units in 100 µl) and subjected to the same procedure as described above. As the positive control, gentamicin was used at concentrations of 250, 125, 62.5, 31.25, 15.62, 7.81 µg/ml. All experiments were performed in triplicate.

Disc Diffusion Technique

Disc diffusion method [17] was employed for screening antibacterial activities of the HWE and

the CEE. Briefly, a suspension of each bacterial strain (100 µl of 1 x 10^5 CFU/ml) was spread on blood agar plates prepared as described previously. Sterile discs (6 mm in diameter) impregnated either with 100 µL of the stock solutions (1000 µg/ml) which equals to 100 µg/disc of the HWE, CEE or gentamicin (as the positive control) were placed on the inoculated blood agar plates. The test plates were incubated at 37 ^oC for 24 h and visually examined for a visible growth of the bacterial strains. After 24 h, the diameters of the inhibition zones were measured in millimeters. To detect whether the extract could mediate dose dependant change, sterile discs (6 mm in diameter) containing different doses of the HWE, CEE or gentamicin (100 µg/disc – 12.5 µg/disc) were placed on the surface of the blood agar plate and subjected to the same experimental procedure as mentioned above. All the experiments were performed in triplicate.

Statistical Analysis

Data are given as means ± S.E.M. Statistical comparisons were made using one way ANOVA followed by Duncans Multiple range test. A *P* value ≤ 0.05 was considered as significant.

RESULTS

Effects of the HWE and the CEE on viable colony count

As evident from (**Table 1**) both HWE and CEE demonstrated significant ($P \leq 0.05$) antibacterial effects against *S. aureus, S. pyogenes, E. coli* and *P. aeroginosa.* On comparing the two extracts against the tested bacterial strains, the CEE was found to be more effective as an antibacterial agent than the HWE. Thus, a concentration of 56.25 µg/mL of HWE and 28.12 µg/mL of CEE required to completely inhibit the growth of above tested organisms. Further, *S. aureus* and *S. pyogenes* appear to be more susceptible than *E. coli* and *P. aeroginosa* to the antibacterial actions of the HWE and CEE. However, the positive control, gentamicin was found to be a more potent antibacterial agent than both CEE and HWE.

Table 1. Antibacterial activity of the hot water extract (HWE) and the cold ethanolic extract (CEE) of *Trichosanthes cucumerina* by viable colony count assay

Type of extract	Concentrations (µg/ml) of *Trichosanthes cucumerina* extracts that completely inhibited growth of Bacterial strain			
	E. coli	***P. aeroginosa***	***S. aureus***	***S. pyogenes***
Hot water Extract(HWE)	56.25 ± 0^{a}	56.25 ± 0^{a}	28.12 ± 0^{a}	28.12 ± 0^{a}
Cold Ethanolic Extract (CEE)	28.12 ± 0^{b}	28.12 ± 0^{b}	14.06 ± 0^{b}	14.06 ± 0^{b}
Gentamycin	7.03 ± 0^{c}	7.03 ± 0^{c}	7.03 ± 0^{c}	7.03 ± 0^{c}

Values are expressed as mean ± S.E.M., n = 3,a – Significantly ($P \leq 0.05$) different from b and c,b – Significantly ($P \leq 0.05$) different from a and c, c – Significantly ($P \leq 0.05$) different from a and b

Effects of the HWE and the CEE on Disc Diffusion

As shown in (**Table 2**) both HWE and CEE showed significant ($P \leq 0.05$) antibacterial effects against *S. aureus, S. pyogenes, E. coli* and *P. aeroginosa* in the disc diffusion assay. When comparing the two extracts against the tested bacterial strains, the CEE demonstrated greater antibacterial activity than the HWE. As in the colony count method, *S. aureus* and *S. pyogenes* appear to be more susceptible than *E. coli* and *P. aeroginosa* for the antibacterial actions of the HWE and CEE. The positive control, gentamicin was found to be a more potent antibacterial agent than both CEE and HWE.

Table 2. Antibacterial activity of the hot water extract (HWE) and the cold ethanolic extract (CEE) of *Trichosanthes cucumerina* by disc diffusion assay

Bacterial strains	Diameter of inhibition zones (in mm)		
	Cold ethanolic extract (CEE) 50 µg/disc	**Hot water extract (HWE) 50 µg/disc**	**Gentamicin 50 µg/disc**
S. aureus	16.8 ± 1.1^{a}	20.3 ± 0.6^{a}	39.2 ± 2.3^{a}
S. pyogenes	18.2 ± 0.8^{a}	22.7 ± 0.9^{a}	40.8 ± 0.6^{a}

E. coli	26.3 ± 0.8 [b]	30.8 ± 1.1 [b]	45.9 ±1.1 [a]
P. aeroginosa	24.5 ± 1.2 [b]	28.3 ± 0.8 [b]	43.5 ± 1.4 [a]
	25 µg/disc	25 µg/disc	25 µg/disc
S. aureus	10.4 ± 1.1 [a]	15.8 ± 0.4 [a]	35.8 ± 0.8 [a]
S. pyogenes	12.7 ± 0.4 [a]	18.4 ± 0.3 [a]	35.1 ± 0.4 [a]
E. coli	17.8 ± 0.5 [b]	25.3 ± 0.6 [b]	40.9 ±1.3 [a]
P. aeroginosa	22.5 ± 1.2 [b]	28.3 ± 0.8 [b]	40.5 ± 1.1 [a]
	12.5 µg/disc	12.5 µg/disc	12.5 µg/disc
S. aureus	4.8 ± 0.8 [a]	8.6 ± 0.6 [a]	30.5 ± 1.2 [a]
S. pyogenes	6.8 ± 1.1 [a]	10.6 ± 0.5 [a]	28.3 ± 2.2 [a]
E. coli	12.5 ± 0.9 [b]	18.6 ± 1.1 [b]	35.7 ± 2.2 [a]
P. aeroginosa	17.5 ± 0.7 [b]	21.6 ± 1.2 [b]	36.5 ± 0.9 [a]
	6.25 µg/disc	6.25 µg/disc	6.25 µg/disc
S. aureus	0.0 ± 0.0	0.0 ± 0.0	34.6 ± 0.9 [a]
S. pyogenes	0.0 ± 0.0	0.0 ± 0.0	30.5 ± 1.5 [a]
E. coli	0.0 ± 0.0	0.0 ± 0.0	27.5 ± 1.1 [a]
P. aeroginosa	0.0 ± 0.0	0.0 ± 0.0	20.8 ± 1.4 [a]

Values are expressed as mean ± S.E.M., n = 3, a – Significantly ($P \leq 0.05$) different from b , b – Significantly ($P \leq 0.05$) different from a

DISCUSSION

The activity of plant extracts against bacteria has been studied for years, but more aggressively during the last three decades [18]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infections agents has led to the screening of several medicinal plants for their potential antimicrobial activity [19]**.**The present study reveals that *T. cucumerina* has components that can exert significant antibacterial activity against *S. aureus, S. pyogenes, E. coli* and *P. aeroginosa* that are known well as wound pathogens [2]. Furthermore, the CEE can exert consistently better antibacterial activity than the HWE. This difference in activity of the HWE and CEE may be related to the polarity of antibacterial compounds present in *T. cucumerina.* Some other medicinal plants have also been reported to have strong antibacterial effects in organic extracts than aqueous extracts. Examples include *Catharanthus roseus* [20] and *Aporosa lindleyana*[21] .

A zone of inhibition≥ 9-15 mm is an indication of strong antimicrobial activity [22]. *E. coli* and *P. aeroginosa* were found to be most susceptible to *T. cucumerina* with an inhibition zone greater than 9 mm at a very low concentration (12.5 µg/disc) of both extracts. Phytochemical constituents such as tannins, saponins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and other herbivores [23]. Phytochemical investigations reveal that *T. cucumerina* extracts also contain tannins, saponins, flavonoids and alkaloids as major chemical constituents [15]. Therefore, these phytochemicals may contribute to its antibacterial activity. Both *E. coli* and *P. aeroginosa* were found to be more susceptible than *S. aureus* and *S. pyogenes* to the action of the *T. cucumerina* extracts. This is possibly due to the differences in chemical composition and structure of cell wall of the microorganisms.

In conclusion, *T. cucumerina* extracts exhibited antibacterial activity against both gram (+) ve bacterial strains such as *S. aureus, S. pyogenes* and gram (-) ve bacterial strains such as *E. coli* and *P. aeroginosa* mediating the presence of a broad spectrum of antibacterial compounds in the plant.

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