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Anti-bacterial Activity of Aqueous and Methanolic Root Extracts of Medicinal Plant *Heteropogon contortus*

Ruvani Gamagea, Buddhika Hasanthia,*, Rajith Silvab, Daya Ratnasooriyab, Ranjith Pathiranab, Sujeewa Hettihewac,

a National Science Foundation, Maitland Place, Colombo 7, Sri Lanka; b Department of Basic Sciences, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Werahera, Sri Lanka; cDepartment of Pharmacy, Faculty of Medicine, University of Ruhuna, Karapitiya, Sri Lanka

ABSTRACT

Synthetic drugs available for the treatment of chronic and infectious diseases are very expensive and some of them have become less effective due to the emergence of antibiotic resistance. Researchers are now focused on natural sources including plants and plant parts for the development of novel therapeutic agents. Therefore, this study evaluated in vitro anti-bacterial activity and phytochemical composition of aqueous and methanolic root extracts of Sri Lankan medicinal plant Heteropogon contortus against clinically significant pathogens, Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923). Agar well diffusion method and standard chemical tests were performed to evaluate anti-bacterial effect and phytochemical profile of the root extracts, respectively. The results revealed that each crude root extract exhibited anti-bacterial activity against both E. coli and S. aureus. The largest zones of inhibition against both E. coli and S. aureus were detected from the methanolic root extract at a concentration of 200 mg/mL and this is a novel finding. Phytochemical analysis of roots revealed the presence of bioactive constituents that account for the anti-bacterial potential, such as alkaloids, phenols and flavonoids. Thereby, root of H. contortus is a reliable source to develop potent, cheap and natural anti-bacterial agents. Future research focusing on high concentrations of different root extracts is recommended to further understand the anti-bacterial capability of H. contortus.

Keywords: Heteropogon contortus, medicinal plant, root, anti-bacterial activity

***Correspondence to Author:**
Buddhika Hasanthi
National Science Foundation, Maitland Place, Colombo 7, Sri Lanka

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Introduction

Medicinal plants can be used in the development of new drugs as they contain diverse natural compounds owing therapeutic properties (1). As estimated by the WHO, more than 80% of the world population depends on herbal medicine for their primary health care requirements (2). Natural products of the medicinal plants can be used as a novel source of antibiotics to address the increasing therapeutic problem, anti-microbial resistance (3). Herbal medicines obtained from higher plants are more popular because they have minimal side effects compared to the synthetic drugs and also due to their frequent availability and low cost. The development of novel antibiotics which are able to combat against the anti-microbial resistance developed by most of the pathogenic bacteria like methicillin resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* is an urgent need to treat most of the infectious diseases (4).

Hence, this study was carried out to investigate the anti-bacterial efficacy of the root of the Sri Lankan medicinal plant *Heteropogon contortus* against two clinically significant pathogenic strains such as, *S. aureus* and *E. coli*. *H. contortus* is a perennial grass found in Southern Asia, Southern Africa and Northern Australia and commonly known as spear grass or black spear grass. It belongs to the family Poaceae and has densely tufted and slender stems compressed towards the base. Stems branched above ground at flowering. Leaves and sheaths are green to grey-green, leaf blades are linear, 3-30 cm long, 2-8 mm wide, abruptly narrowed at the tip and basal sheaths are laterally compressed (5,6). The roots of the plant is used in Sri Lankan traditional medicine as a diuretic, and also used to treat asthma, bronchial diseases, jaundice, ulcers, wounds and cystitis (5). The broncho relaxant activity and anti-inflammatory activity of the plant and the quantification of lupeol from leaves, stem and inflorescence of plant have been well established (7, 8).

Even though the roots of *H. contortus* are used in traditional Sri Lankan medicinal remedies (5), their therapeutic potential, particularly anti-bacterial efficacy, has not been scientifically well-proven indicating that this topic is poorly studied to date. Thereby, present study was conducted to evaluate the *in vitro* antibacterial effect of the aqueous and methanolic extracts of the root *H. contortus* and to evaluate the phytochemical composition of those root extracts.

Materials and methods

Collection of the root *H. contortus*

The fresh plants of *H. contortus* were collected from Galle district (6.0535° N; 80.2210° E), Sri Lanka in June, 2017.

Authentication of plant material

The plant material was identified at the Department of Plant Sciences, Faculty of Sciences, University of Colombo, Sri Lanka. A voucher specimen was deposited at the Department of Basic Sciences, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Sri Lanka.

Extracts preparation for the plant *H. contortus*

Fresh whole plants of *H. contortus* were taken and roots were separated. Roots were washed twice with normal saline. Then the roots were dried under the shade until a constant weight was obtained. The roots were powdered using a domestic blender. Powdered roots (10.0 g each) were mixed with 50 mL of distilled water and methanol, separately and subjected to extraction for 7 days. Each extract was filtered through a Whatman No.1 filter paper. Filtrates were then stored in sealed beakers and kept in a refrigerator (4^0C) until use.

Test microorganisms

Pathogenic strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were obtained from Medical Research Institute, Colombo 08, Sri

Lanka and were maintained on Nutrient agar slants at 4^0C for further experiments.

Preparation of inoculums

Two culture plates were prepared for the above-mentioned two organisms from the slants. Bacterial colonies from each organism were added to saline water and bacterial suspensions equivalent to 0.5 McFarland standard were prepared for both *E. coli* and *S. aureus.*

Agar well diffusion method

Four metal cylinders with a diameter of 8 mm were kept on the petri-dishes with same distances and agar was poured. After settling down, the cylinders were carefully removed and the agar plate surface was inoculated by spreading 200 µL of microbial suspension over the entire agar surface. Each agar plate was then introduced with 100 µL of two dilutions (200 mg/mL and 100 mg/mL) of each extract, positive and negative controls. 1 µg/mL Gentamycin was used as the positive control, whereas distilled water and methanol were used as the negative controls depending on the type of extract tested. This procedure was performed separately in triplicates for the microbial suspensions of *E.coli* and *S.aureus*. All agar plates were incubated at 37^0C for 24 hours.

Phytochemical analysis of the aqueous and methanolic root extracts

Aqueous and methanolic root extracts of *H. contortus* were subjected to following phytochemical screening. Qualitative analysis was performed for alkaloids using Meyer's and Wagner's tests. Test for phenols was achieved via Libermann Burchard's test and $FeCl_3$ test. Alkaline reagent test was carried out for flavonoids. Additionally, foam test was performed for the analysis of saponins. Molish test and xanthoproteic test were performed for the investigation of carbohydrate and proteins, respectively. Gelatin test was carried out for tannins.

Statistical analysis

The results were given as mean ± SEM. Data analysis was performed by SPSS version 21.0. Statistical comparisons were made using Duncan's new multiple range test. Significance was set at $P<0.05$.

Results

In vitro antibacterial activity of aqueous and methanolic root extracts of *H. contortus* was tested using agar well diffusion method. Anti-bacterial activity of root extracts against *E. coli* was tabulated in the Table 1.

Table 1: Antibacterial activity of aqueous and methanolic root extracts of medicinal plant *H. contortus* against *E. coli*

Extracts	Diameter of the zone of inhibition in mm (Mean ± SEM)			
	200 mg/mL	100 mg/mL	Negative control	Gentamycin 1µg/mL
Aqueous	13.93±0.23	11.90±0.10	8.06±0.07	28.33±0.33
Methanol	15.10±0.06	13.40±0.05	8.33±0.33	27.97±0.17

SEM = Standard error mean

Among the two extracts tested, the highest zone of inhibition against *E. coli* was exhibited by the 200 mg/mL methanol extract. Both concentrations of methanolic root extract showed significant inhibition against *E. coli* when compared to the respective negative control (P<0.05). Similarly, both 200 mg/mL and 100 mg/mL concentrations of aqueous root extracts also exhibited significant inhibitions against *E. coli* compared to the respective negative control (P<0.05), but the zones of inhibition is lesser than the respective concentrations of methanolic root extracts.

As shown in Table 2, 200 mg/mL methanolic root extract was able to exhibit the highest zone of inhibition against *S. aureus* and it was significant compared to the respective negative control (P<0.05). 200 mg/mL concentration aqueous extract also exhibited a significant inhibition (P<0.05), whereas, among 100 mg/mL concentrations, only methanolic root extract exhibited a significant inhibition (P<0.05) compared to the respective negative control.

Table 2: Antibacterial activity of aqueous and methanolic root extracts of medicinal plant *H. contortus* against *S. aureus*

Extracts	Diameter of the zone of inhibition in mm (Mean ± SEM)			
	200 mg/mL	100 mg/mL	Negative control	Gentamycin 1µg/mL
Aqueous	12.20±0.20	10.33±0.33	8.20±0.20	28.33±0.33
Methanol	16.30±0.15	14.40±0.21	8.06±0.07	32.23±0.12

Gentamycin, the positive control, showed the largest zones of inhibition against both *E. coli* (P<0.05) and *S. aureus* (P<0.05) compared to the negative control used in this study. Further, all extracts showed a significantly different inhibition compared to Gentamycin (P>0.05). This result indicated that although the tested extracts possess *in vitro* antibacterial activity against both *E. coli* and *S. aureus*, the activity is not comparable with the reference drug, Gentamycin.

The results of the phytochemical analysis of aqueous and methanolic root extracts of *H. contortus* were depicted in Table 3.

Table 3: Phytochemical analysis of aqueous and methanolic root extracts of medicinal plant *H. contortus*

Chemical class	Test performed	Response	
		Methanol	Aqueous
Alkaloids	Meyer's test	**+**	**+**
	Wagner's test	**+**	**+**
Phenols	Libermann's test	**+**	**-**
	Neutral $FeCl_3$ test	**+**	**+**
Flavonoids	Alkaline reagent test	**+**	**-**
Saponins	Foam test	**-**	**-**
Carbohydrates	Molish test	**+**	**+**
Proteins	Xanthoproteic test	**+**	**+**
Tannins	Gelatin test	**-**	**-**

+ = Positive response; **-** = Negative response

Discussion

At present, herbal plants are explored as possible sources for novel anti-microbial, such as anti-bacterial agents due to the emergence of anti-bacterial resistance (9). In this view, this study assessed *in vitro* anti-bacterial activity and phytochemical profile of aqueous and methanolic root extracts of Sri Lankan medicinal plant *H. contortus*. This study has utilized agar well diffusion method to evaluate *in vitro* anti-bacterial activity and standard chemical tests for the phytochemical screening. The experimental conditions, setup and bioassays used in this study were well-recognized and widely used by several other investigators (10,11,12).

E. coli and *S. aureus* were used as test microorganisms because of their clinical importance. *E. coli* is the leading cause for urinary tract infection (UTI) and the most common Gram-negative pathogenic strain in humans (13,14). Additionally, occurrence of MRSA stains is rapid in the world and Asia has been recognized as the region with highest incidence of MRSA (15). Results of this study indicated that both aqueous and methanolic root extracts of medicinal plant *H. contortus* exhibited *in vitro* anti-bacterial activity against *E. coli* and *S. aureus*.

In this study, the diameter of the zone of inhibition was taken into consideration for the assessment of *in vitro* anti-bacterial activity. Similarly, Balouiri *et al*. (2016) have stated that the efficiency of antibacterial activity of a plant extract is usually expressed by its diameter of zone of inhibition in which higher the diameter, the more effective is the agent as an anti-bacterial (16).

From the extracts tested, the highest anti-bacterial activity was exhibited by the methanolic root extract against *S. aureus* at a concentration of 200 mg/mL and the diameter of zone of inhibition was 16.30±0.15 mm. The highest anti-staphylococcal activity expressed by methanolic root extract in this study is noteworthy because of the emergence of MRSA strains and it is a novel finding. Likewise, for *E. coli*, the largest zone of inhibition was also showed by the 200 mg/mL concentration of methanolic root extract indicating the highest anti-bacterial activity when compared to the aqueous root extracts. Yet, both concentrations of aqueous root extracts also exhibited anti-bacterial effect against *E. coli* and *S. aureus*.

Findings of this study signified that the anti-bacterial effect observed for both *E. coli* and *S. aureus* is genuine and intrinsic. Both concentrations of aqueous, expect 100 mg/mL concentration, and methanolic root extracts showed a significant activity compared to the respective negative controls. Nevertheless, this effect is not comparable to the reference dug, Gentamycin, for the tested concentrations. The higher concentrations of each extract may possess more potent anti-bacterial activity and therefore, there is a high possibility exists to develop efficacious and affordable anti-bacterial agents from *H. contortus* roots.

There was no published evidence in the existing literature to support the finding of this study as no other published study was conducted to evaluate the anti-bacterial activity of the roots of *H. contortus*. However, previous studies have also reported the potent *in vitro* anti-bacterial activity of plant extracts that are belonging to family Poaceae.

In their study, Amber *et al*. (2017) have evaluated the anti-bacterial activity of selected medicinal plants including *Oryza sativa* and *Triticum aestivum* belongs to the family Poaceae against both Gram-negative and Gram-positive bacterial pathogens namely *E. coli*, *Klebsiella pneumonia* and *S. aureus*. Results of this study indicated that crude methanolic extracts of both *O. sativa* and *T. aestivum* have expressed a potent anti-bacterial effect against *E. coli*, *K. pneumonia* and *S. aureus* (17). Thus, anti-bacterial potency of methanolic extracts of plats in family Poaceae is also reported in previous research as evident in this study regardless of the type of plant species used. Importantly, in Asian context, medicinal plants and plant parts that belong to the family Poaceae have been used in ethnomedicinal preparations and ethnoveterinary practices since ancient times due to their potent anti-bacterial properties (17,18,19).

Regardless of not scientifically well-proven anti-bacterial activity of *H. contortus*, aqueous root extracts of *H. contortus* have been used to treat broad-spectrum of bacterial induced infections including wounds, ulcers and diarrhea (5) in rural parts of Sri Lanka. In this context, the potent anti-bacterial activity expressed by the aqueous root extract of *H. contortus* is very vital as this study enhances the scientific validity of the above-mentioned ethnomedicinal practice. In addition to its use in human infections, some of the traditional medicine books have also reported the use of *H. contortus* roots in ethnoveterinary practices in Sri Lanka for the treatment of microbial infections in animals, particularly in cows and buffalos. Hence, the application of *H. contortus* roots in ethnomedicinal and ethnoveterinary practices as a cure for human and animal diseases can be explained by its anti-bacterial property. The biologically active natural compounds present in the roots of *H. contortus* mainly account for the anti-bacterial effect.

Anti-bacterial property exhibited by the root of *H. contortus* is attributed to the phytoconstituents present in them. Phytochemical analysis of roots revealed the presence of several bioactive constituents. Particularly, aqueous root extract contained alkaloids, phenols, carbohydrates and proteins, whereas methanolic root extract contained alkaloids, phenols, flavonoids, carbohydrates and proteins. Among those compounds, alkaloids, phenols and flavonoids are well-recognized for their potent anti-bacterial activity (20,21,22).

Naturally occurring alkaloids are able to inhibit sortase A in Gram-positive bacteria. Sortases are enzymes that attach certain essential proteins, such as adhesins, internalins and immune evasion, to the Gram-positive bacterial cell walls. Further, alkaloids can disrupt fimbriae and other adhesions via non-sortase mediated mechanisms as well (23), which result in distrupting the signaling pathways and physiological activities of Gram-positive bacteria (24). Alkaloids also possess anti-bacterial potential which is mediated via interactions with bacterial DNA (25). Phenolic compounds are capable of obstructing peptidoglycan synthesis in bacterial cell wall, alter surface hydrophobicity of bacterial membrane, destruct bacterial membrane structures and change the signal transduction (9,26). Flavonoids are formed in plants in response to various microbial infections and they demonstrate inhibitory activities against bacteria, fungi and viruses (27). Consequently, root extracts of *H. contortus* tend to demonstrate anti-bacterial potency against *E. coli* and *S. aureus* via single or multiple above-mentioned mechanisms as evident in this study.

In this present study, distilled water and methanol were used as solvents to extract biologically active compounds present in the roots of *H. contortus*. For both microorganisms, methanolic root extract at a concentration of 200 mg/mL showed the highest anti-bacterial activity by exhibiting the largest zones of inhibition when compared to the aqueous root extracts. Therefore, it can be said that methanol is a good solvent than distilled water to extract biologically active compounds that are responsible for the anti-bacterial potential of *H. contortus* roots. Similarly, previous research have also identified methanol as a suitable solvent to extract many of the active components present in plants, such as anthocyanins, terpenoids, saponins, tannins, xanthoxyllines, totarol, quassinoids, lactones, flavones, phenones and polyphenols (28), than distilled water. Phytochemical analysis of this study also showed that flavonoids are present in the methanolic root extracts but not in the aqueous extracts indicating that methanol is more appropriate than distilled water to extract many of the naturally occurring organic compounds. This could be the reason for greater anti-bacterial activity exhibited by methanolic root extract as it contains many of the biologically active compounds that are responsible for the anti-bacterial potential.

Conclusion

In conclusion, present study showed *in vitro* anti-bacterial potential of aqueous and methanolic root extracts of *H. contortus* and it is mediated via biologically active compounds, phytochemicals, present in the root. Study findings suggest that *H. contortus* roots can be used to develop promising, safe and relatively cheap anti-bacterial agents. Future research focusing on high concentrations of root extracts and evaluation of minimum inhibitory concentrations (MICs) are recommend to better understand the anti-bacterial efficacy of the root *H. contortus*.

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