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In vitro anti-bacterial activity of aqueous extracts of the whole plant, flowers, roots, leaves and stem of grass *Heteropogon contortus* grown in Sri Lanka

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Abstract
In vitro antibacterial potential of aqueous extracts of *Heteropogon contortus* (whole plant, leaves, flowers, stem and roots) were examined against gram negative and positive pathogen; *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) respectively. Concentrations tested were 100 and 200 mg/mL, using agar well diffusion bioassay technique. Gentamicin (1μg/mL) was used as the positive control and distilled water as the negative control. The results revealed that different parts of the plant exhibit different degrees of antibacterial activity. The ranking of the potency of antibacterial activity against *Escherichia coli* was whole plant > leaves > flowers > root > stem, while whole plant > flowers > roots > leaves > stem for *Staphylococcus aureus*. Phytochemical analysis revealed the presence of alkaloids, flavonoids, diterpenes and glycosides in certain parts of the plant while phenols, proteins and amino acids were found in all parts. The antibacterial activity is likely to be mediated by synergistic action via multiple mechanisms. A strong possibility exists to develop a safe and potent antibacterial agent from *Heteropogon contortus.*

Keywords: *Heteropogon contortus*, antibacterial activity, *in vitro*, extracts.

1. Introduction

Infectious diseases have become the world's foremost reason of morbidity and mortality specially in children which kills nearly 50 000 people every day due to the drug resistance developed by the human pathogenic bacteria all over the world (Ahamed and Beg, 2001; Armando *et al*., 1991) [1]. Particularly, with unselective use of broad-spectrum antibiotics, intravenous catheters, immunosuppressive drugs and ongoing epidemics of HIV infection, the occurrence of multi-drug resistance bacteria is continuously increasing. Furthermore, high cost and the scanty of the synthetic drugs in developing countries also contribute to this issue (Dabur *et al*., 2007) [8]. Therefore, there is an urgent necessity to find out novel antibiotics with new drug action and varied chemical structures to overcome this resistance and the re-emerging infectious diseases (Rojas *et al*., 2003) [18]. With that there is an increased attention paid towards the folk medicine to develop better antimicrobial drugs (Sukanya *et al*., 2009) [21].

Medicinal plants and their natural products represent a novel source for antimicrobial agents. They can modify many of the side effects that are often related with the synthetic antimicrobial agents with their involvement in the treatment of infectious diseases (Shihabudeen *et al*., 2010) [19]. The different parts of the plants like flower, stem, root, twigs exudates, fruit and modified plant organs are used to extract raw drugs with varied medicinal properties. Even though hundreds of plant species have been screened against the antimicrobial activity, only a small percentage has been tested phytochemically (Mahesh and Sathish, 2008) [13] Considering those gaps in the existing literature, this study was carried out to investigate the antibacterial activity and phytochemical profile of the medicinal plant *Heteropogon contortus* (*H. contortus*). *H. contortus* is a perennial grass belongs to family Poaceae with densely tufted and slender stems compressed towards the base. Stems branched above ground at flowering. Leaves and sheaths green to grey-green, leaf blades are linear, 3-30 cm long, 2-8 mm wide and abruptly narrowed at the tip and basal sheaths laterally compressed (Ayurvedic Medicinal Plants of Sri Lanka; Jayaweera, 1981) [3, 11].

The grass is commonly known as spear grass, black spear grass and bellary grass and can be found in Southern Asia, Southern Africa and Northern Australia. Roots of the plant have diuretic and stimulant action and the plant has been also used in the treatment of asthma, bronchial diseases, jaundice, toothache, fever, dysentery, muscular pain and scorpion sting (Ghante *et al*., 2013; Jayaweera, 1981) [10, 11]. 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 (Ghante *et al*., 2013; Kaur and Gupta, 2017) [10, 12]. In present investigation, an attempt has been taken to investigate the antimicrobial activity of the aqueous extracts of whole plant, leaves, flowers, stem and roots of the Sri Lankan grass *H. contortus* against clinically important two pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli* and to evaluate the phytochemical composition of the plant.

2. Materials and methods

2.1. Collection and authentication of plant material

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

2.2. Extracts preparation for the plant *H. contortus*

Fresh whole plants of *H. contortus* were taken and separated into different parts as leaves, flowers, stem and roots. Parts of the plants were washed twice with normal saline. They were crushed using a mortar and pestle separately. Ten gram of each part and the 40 g of the whole plant (10 g of each leaves, flowers, stem and roots) were subjected to extraction with 50 mL and 200 mL of distilled water for 7 days, respectively. Each extract was filtered through a Whatman No.1 filter paper. Filtrates were then stored in sealed beakers and kept in a refrigerator (4 ^{0}C) until use.

2.3. Test microorganisms

Pathogenic strains of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were obtained from Medical Research Institute, Colombo 08, Sri Lanka and were maintained on Nutrient agar slant at 4 ^{0}C for further experiments.

2.4. 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.*

2.5. 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 loaded with 100 µL of two dilutions (200 mg/mL and 100 mg/mL) of each extract, positive and negative controls. 1 µg/mL Gentamicin was used as the positive control, whereas distilled water was used as the negative control. This procedure was performed separately in triplicates for both microbial suspensions of *E. coli* and *S. aureus*. All agar plates were incubated at 37 ^{0}C for 24 hours and the diameter of the zone of inhibition (in mm) of each extract against two bacterial species were measured and recorded.

2.6. Phytochemical analysis

Aqueous extracts of the whole plant and different parts of *H. contortus* were subjected to following phytochemical Screening. Qualitative analysis was performed for alkaloids using Mayer's, Dragendroff's and Wagner's tests. Molish, Benedict's and Felling's tests were performed to detect carbohydrates. $FeCl_3$ and modified Borntrager's tests were performed for the analysis of phenols and glycosides, respectively. Alkaline reagent test was carried out to test flavonoids. Saponins and phytosterols were tested using foam test and Libermann Burchard's test, respectively. For the investigation of tannin, gelatin test was performed. Additionally, xanthoproteic and ninhydrin tests were performed for the analysis of proteins and amino acids, respectively. Copper acetate test was performed to investigate the presence of diterpenes.

2.7. 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 $P<0.05$.

3. Results

Each extract of the whole plant and different parts of *H. contortus* were tested for the antibacterial activity using agar diffusion method. *In vitro* antibacterial activity of the extracts against *E. coli* is tabulated in the Table 1.

Table 1: Antibacterial activity of the aqueous extracts of the plant *H. contortus* against *E.coli* (Mean±SEM)

Diameter of the zone of inhibition in mm (Mean±SEM)				
Extract	**200 mg/mL**	**100 mg/mL**	**Negative control**	**Gentamicin 1µg/mL**
Leaf	18.00±0.58	15.33±0.33	8.06±0.07	29.67±0.33
Stem	8.33±0.33	8.10±0.03	8.33±0.33	30.33±0.33
Root	13.93±0.23	11.90±0.10	8.06±0.07	28.33±0.33
Flower	15.50±0.29	12.20±0.20	8.33±0.33	31.67±0.33
Whole plant	24.33±0.33	16.30±0.30	8.13±0.03	35.87±0.44

SEM= standard error of mean

Among the extracts of the different parts, the highest zone of inhibition against *E. coli* was exhibited by the 200 mg/mL leaf extract. Both concentrations of the leaf extract showed significant inhibition against *E. coli* when compared to the negative control ($P<0.05$). 200 mg/mL and 100 mg/mL concentrations of root and flower extracts also exhibited significant inhibitions against *E. coli* ($P<0.05$), whereas both concentrations of the stem extract did not exhibit significant inhibition compared to the negative control ($P>0.05$). Whole plant extracts at concentrations of 200 mg/mL and 100 mg/mL showed significant inhibitions compared to the negative control ($P<0.05$) and those zones of inhibition were greater than the inhibitions of the respective concentrations of the extracts of different parts. There are no significant differences between the inhibitions exhibited by the both concentrations of leaf extracts and the respective concentrations of whole plant extract (P>0.05) while there is a significant difference between the stem, root and flower extract with the whole plant extract (P<0.05) against *E. coli*.

Table 2: Antibacterial activity of the aqueous extracts of the plant *H.contortus* against *S.aureus* (Mean±SEM)

Extracts	Diameter of the zone of inhibition in mm (Mean±SEM)			
	200mg/mL	100mg/mL	Negative control	Gentamicin 1μg/mL
Leaf	10.33±0.33	8.33±0.33	8.06±0.06	32.33±0.33
Stem	11.80±0.14	10.17±0.17	8.33±0.33	28.33±0.33
Root	12.20±0.20	10.33±0.33	8.20±0.20	28.33±0.33
Flower	20.67±0.33	17.83±0.33	8.33±0.33	31.67±0.33
Whole plant	22.5±0.50	18.10±0.10	8.13±0.03	29.67±0.33

SEM= standard error of mean

As indicated in Table 2, the highest zone of inhibition for *S. aureus* was exhibited by the 200 mg/mL concentration of the flower extract which is significant with the negative control (P<0.05) and the 100 mg/mL concentration of the same extract also inhibited *S. aureus* with a significant zone of inhibition (P<0.05). 200 mg/mL concentrations of bark and root extracts also exhibited antibacterial activity against *S. aureus* which are significant compared to the negative control (P<0.05). However, both concentrations of the leaf extracts did not inhibit the growth of *S. aureus* significantly (P>0.05). The inhibitions of the both 200 mg/mL and 100 mg/mL extracts of the whole plant are greater than the inhibitions of the parts of the plant and they are significant compared to the negative control (P<0.05). There are no significant difference between the inhibitions exhibited by the both concentrations of flower extracts with the respective concentrations of the whole plant extract (P>0.05), while there is a significant difference between the leaf, stem and root extracts with the whole plant extract (P<0.05) against *S. aureus*.

Gentamicin, the positive control, showed the largest zones of inhibition against both *E. coli* (P<0.001) and *S. aureus* (P<0.05) compared to the negative control used in this study. Further, all extracts showed a significant difference of inhibition compared to Gentamicin (P>0.05). This result indicated that although the tested extracts possess *in vitro* antibacterial activity against *E. coli* and *S. aureus*, the activity is not comparable with the reference drug, Gentamycin.

The results for the phytochemical analysis of the whole plant and different plant extracts were tabulated in the Table 3.

Table 3: Phytochemical analysis of aqueous extracts of the different parts of *H. contortus*.

Chemical class	Test performed	Leaves	Stem	Root	Flower	Whole plant
Alkaloids	Meyer's test	-	-	+	+	+
	Dragendroff's test	-	-	+	+	+
	Wagner's test	-	-	+	+	+
Carbohydtrates	Molish test	+	+	+	+	+
	Benedict's test	-	+	-	+	+
	Felling's test	-	+	-	+	+
Glycosides	Modified Borntragers test	+	+	+	+	+
Saponins	Foam test	-	-	-	-	-
Phytosterols	Libermann Burchard's test	-	-	-	-	-
Phenols	Ferric Chloride test	+	+	+	+	+
Tannin	Gelatin test	-	-	-	+	+
Flavanoids	Alkaline reagent test	+	-	-	-	+
Proteins and	Xanthoproteic test	+	+	+	+	+
amino acids	Ninhydrin test	+	+	+	+	+
Diterpenes	Copper acetate test	-	-	-	+	+

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

4. Discussion

Plant and plant extracts have been used in Sri Lankan traditional medicine since ancient times due to their promising curative properties (Ayurvedic Medicinal Plants of Sri Lanka; Jayaweera, 1981) [3, 11]. However, the therapeutic potential of most the plant extracts used in folk medicine has not been scientifically well-proven and documented to date. In this context, antibacterial activity of aqueous extracts of the whole plant and leaf, flower, stem root of *H. contortus* were tested against two pathogenic microbes viz., *E. coli*, a normal inhabitant of human, which some strains can cause intestinal and extra-intestinal infections like gastroenteritis, urinary tract infections, septicemia and neonatal meningitis (Clermont *et al*., 2000) [6], *S. aureus* a most important pathogen which cause nosocomial infections like pneumonia, surgical wound infections and septicemia (Panlilio *et al*., 1992) [16].

The results of this study revealed that aqueous extracts of the whole plant and different parts of *H. contortus* possess varying degrees of *in vitro* antibacterial activity against both Gram negative, *E. coli*, and Gram positive, *S. aureus*, bacteria as a novel finding. Among the individual extracts tested, the highest antibacterial activity against *E. coli* was expressed by the leaf extract and the strong antimicrobial activity of the whole plant against *E. coli* could be mainly attributed by the activity of leaf. Antibacterial activity of root and flower extracts may also contribute to this. Stem of the plant did not express any antibacterial activity against *E. coli*. Thus, the potency of ranking for antibacterial activity against *E. coli* was whole plant > leaf > flower > root > stem.

This study also showed that whole plant and different parts of *H. contortus* plsses *in vitro* antibacterial activity against *S. aureus*. The highest activity against *S. aureus* was showed by the flower extract. Moreover, leaf, stem and root extracts also displayed antibacterial activity against *S. aureus*. Thus, the potency of ranking for antibacterial activity against *S. aureus* was whole plan > flower > root > leaf > stem.

The antibacterial activity expressed by the whole plant extract could be resulting from the synergistic effect of the antibacterial activity of all tested parts of the plant. The significant antibacterial activity present in the whole plant *H. contortus* against *S.aureus* is very noteworthy as methicillin resistant *Staphylococcus aureus* (MRSA) is very difficult to eliminate due to their emerging resistance to many antibiotics (Enright *et al*., 2002) [9]. Even though *in vitro* antibacterial activity of *H. contortus* is reported for the first times, several other studies have reported the *in vitro* antibacterial activity of the different plant species belong to the family *Poaceae*. For example, antibacterial inhibition of aqueous and ethanol extracts of the leaf *Cynodon dactylon* was reported against *E. coli* (Pandey *et al*., 2016) [15].

Interestingly, the whole plant extract of *H. contortus* expressed a marked and significant antibacterial activity against both *E. coli* and *S. aureus* showing that it can be used as a novel source of antibacterial agent against both Gram-positive and Gram-negative bacteria. This antibacterial activity may be resulting from the synergistic effect of phytochemical constituents present in the different parts of the plant. Similar to the findings of this study, a study conducted in Palestine on selected medicinal plants also revealed the synergistic effect of antibacterial activity of aqueous plant extracts against *E. coli*, *Pseudomonas aeruginosa* and *S. aureus* (Amenu, 2014) [2] as evident in this study.

The exhibited strong antibacterial potential of the aqueous extract of the whole plant is significant because of its application in livestock farming in Asian region. Especially in Sri Lanka, most of the farmers tattoo their animals, mainly cows and buffalos, in order to identify them easily. For this tattooing, farmers usually use heated metal and write owners initials or unique identification symbol on the surface of the animals' skin. Because of this unhygienic cultural practice, most of the times tattooing causes infections. Though it is not scientifically documented, ancient medicine books reported the use of whole plants of *H. contortus* as a cure of those infections. The findings of this study were able to prove the use of *H. contortus* in such farming practices as it has a very rich antibacterial potential.

Phytochemical constituents like alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against many microorganisms, insects and other herbivores (Bonjar *et al*., 2004; Cowan, 1999; Shihabudeen *et al*., 2010) [4, 7, 19]. The present study revealed the presence of medicinally active compounds in *H. contortus* such as alkaloids, tannins, phenols, glycosides, flavonoids, amino acids, diterpenes and carbohydrates which could be responsible for the detected antibacterial property. Except flavonoids, all other above-mentioned compounds were present in the aqueous flower extract as well. Even though the root of *H. contortus* is used for the treatment of diseases like asthma, cystitis and bronchial diseases (Ayurvedic Medicinal Plants of Sri Lanka), the flower of the plant possess the highest antibacterial activity and it is rich with many bioactive compounds. Therefore, there is a great potential exist that flower of *H. contortus* can also be used to treat bacterial infections. Flower extracts of *H. contortus* can be considered for the formulation of novel antibacterial agents in future due to its proven significant antibacterial effect.

The different bioactive compounds exert the antibacterial activity through different mechanisms. Alkaloids possess antibacterial potential by interacting with bacterial DNA (Amenu, 2004; Cowan, 1999) [7]. Tannins bind to proline rich proteins and interfere with bacterial protein synthesis (Shimada, 2006) [20]. Phenols mediate the antibacterial activity via disrupting the functional integrity of the bacterial cell wall and the cell membrane (Pereira *et al*., 2016) [7]. Flavonoids are hydroxylated phenolic substance synthesized by plants in response to microbial infection which can form complex with extracellular and soluble proteins and to complex with bacterial cell walls (Marjorie, 1999) [14]. Diterpenes are an important class of plant metabolites which promote bacterial lysis and disruption of the cell membrane. Accordingly, aqueous extracts of the whole plant and different parts of *H. contortus* may show antibacterial activity against *E. coli* and *S. aureus* via single or multiple above-described mechanisms.

Study findings revealed that *H. contortus* exhibits a promising antibacterial activity against *E. coli* as well as *S. aureus*. Further studies with higher concentration of aqueous plant extracts may leads to discovery of novel antimicrobial drugs. Moreover, the minimum inhibitory concentration (MIC) values of the plant extract should be evaluated. The findings of the present study scientifically confirmed the antibacterial potency and the phytochemical composition of the Sri Lankan grass *H. contortus*. The antibacterial activity expressed by the aqueous extracts of the whole plant and different parts is mediated mostly via phytochemicals present in the plant.

5. Conclusion

This study, for the first time, expressed the *in vitro* antibacterial activity of aqueous extracts of the whole plant as well as different parts of the plant *H. contortus*. Although root is used commonly in medicinal practices, other parts of the plant, especially, the flower has a huge antibacterial potential compared to the root for *S. aureus*. Additionally, leaves demonstrate strong antibacterial activity against *E. coli* when compared to the root. Therefore, there is a strong possibility exists to develop a novel, safe, potent and cheap antibacterial agents from *H. contortus*.

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