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ANALYSIS OF ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL CONTENTS OF A RARE AND ENDEMIC LIANA (*STROPHANTHUS WIGHTIANUS* WALL. EX WIGHT) OF SOUTH INDIA

JOSEPH JOHN[1]*, RAGI, P. R[1], SUJANA, K. A.[2] AND N. ANIL KUMAR[1]

[1]Community Agrobiodiversity Centre, M. S. Swaminathan Research Foundation,Kalpetta, Wayanad, Kerala, India – 673121
[2]Central Botanical Laboratory, Botanical Survey of India, AJCB Indian Botanic Garden, Howrah, West Bengal, India–711103

ABSTRACT

Antibacterial activity of cold water and ethanolic crude extracts of leaves of *Strophanthus wightianus* Wall. ex Wight was evaluated against Gram negative bacteria(*Escherichia coli, Pseudomonas fluorescens, Klebsiella pneumoniae* and *Enterobacter aerogenes*) and Gram positive bacteria (*Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus*, *Streptococcus mutans* and *Bacillus subtilis*). Methicillin resistant *Staphylococcus aureus* was the most sensitive strain of those tested with the cold water extract and *Enterobacter aerogenes* with the ethanolic extract of leaves *Strophanthus wightianus* Wall. ex Wight. Qualitative phytochemical analysis gave positive results for alkaloides, anthraquinones, lipids/fats, glycosides, phenols, carbohydrates, tannins, resins, reducing sugars,saponinsand flavonoids.

KEYWORDS: Antibacterial Activity, Phytochemicals, Rare Liana, *Strophanthus wightianus*, South India

JOSEPH JOHN
Community Agrobiodiversity Centre, M. S. Swaminathan Research Foundation,
Puthoorvayal Post, Kalpetta, Wayanad, Kerala, India – 673121

INTRODUCTION

Natural products are preferred for biological screening based on traditional knowledge of plants, because many communicable diseases are known to have been treated with herbal formulations during the history of mankind. The use of traditional medicines holds a great promise as aeasily available source as effective medicinal agents to cure a wide range of ailments among the peopleparticularly in tropical developing countries like India.Antimicrobial properties of therapeutic plants are being increasingly reported from diverse parts of the world. The Genus *Strophanthus* (Apocynaceae) are best known for the arrow poisons extracted from the crushed seeds, but this group of plants are extremely important ethno-medically distributed in Africa and Asia. The seeds contain a glucoside called strophanthin, which is a cardio-active agent. In some species the ground seeds are used for cardiac insufficiency, while the roots or leaves of other types (e.g. *S. hispidus, S. kombe, S. gratus, S. welwischii, S. preussii,* etc.) are utilized in preparations for venereal diseases, intestinal parasites and serious skin diseases such as scabies [1].

Strophanthus wightianus and *S.wallichi* are endemic to India, out of which *Strophanthus wightianus* Wall. ex Wight, a liana found along the sacred groves of Western Coast of Kerala. Narrow distribution and destruction of natural habitats makes the plant less available for detailed phyto or pharmacological screening. A study conducted by MSSRF to document the rare, endemic and threatened plants of Western Ghats shows that this plant is very rarely seen in scrub juggles and sacred groves of Southern Kerala. The reported uses of the genus *Strophanthus* includes the poisoning of arrows for hunting. A glycoside namely strophanthin is responsible for the poison effect. In this back ground the present study is to screen the phytochemical constituents and antibacterial properties against the selected clinically important pathogens.

MATERIALS AND METHODS

Plant material

The fresh leaves of *Strophanthus wightianus* Wall. ex Wightwere collected from RET plant species conservatory of M. S. Swaminathan Research Foundation, Wayanad, Kerala, India.

Culture and maintenance of bacteria

For the present study antibacterial assay: Gram negative bacteria; *Escherichia coli, Pseudomonas fluorescens, Enterobacter aerogens* and *Klebsiella pneumoniae* and Gram positive bacteria; *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus*, *Streptococcus mutans* and *Bacillus subtilis* were used as test organisms. These microbes were grown in nutrient broth media, and incubated at 37^0C for 48 hrs. Each bacterial culture was further maintained on the same medium after every 48 hours of transferring.

Preparation of plant extracts

Plant materials were surface sterilized and washed with clean sterile water and dried for 1 hour at 160^0C. Three hundred grams of dried plant material was blended to fine powder and soaked in 150 ml of distilled water (cold water extract) and 95 % ethanol (ethanolic extract) for 24 hrs. The slurry obtained was left in clean, sterile glass container and shaken vigorously to allow for proper extraction. The slurry was filtered using a sterile muslin cloth. After which the extract obtained was air dried and stored at 4 ^{0}C until required.

Analysis of antibacterial activity

The filter paper disc method was used for screening of the prepared cold water and ethanolic crude extracts for antibacterial activity. Whatmann filter paper discs (6 mm in

diameter) were sterilized by dry heat sterilization method at 140^0 C for 1 hour. Different dilutions of the plant extract were prepared in the order of 1, 2, 3, 4, and 5 mg/ml respectively. The filter paper discs were saturated with 10 μl of each concentration and known quantity of standard reference antibiotic (Ampicilin) separately. The discs were then placed on the surface of the sterilized nutrient agar media that had been inoculated with test organisms and incubated at 37^0C for 24 hours. The zone of inhibition was considered as an indicator for the antibacterial activity. All the experiments were done in 5 replicates and mean values were computed. Activity Index of test microorganism was calculated by using the formula

$$\text{Activity Index (AI)} = \frac{\text{Inhibition area of the sample}}{\text{Inhibition area of the standard}}$$

Qualitative phytochemical analysis

The ethanolic leaf extracts were analyzed for the pholabatannins, tannins, sterols, lipids, glycosides, terpenoids, phenols, carbohydrates, anthraquinones, resins, reducing sugar, saponins, flavanoids, acidic compounds and alkaloids using standard procedures identify the constituents as described by, Trease and Evans [2] and Harborne[3].

RESULTS AND DISCUSSION

The present study was carried out on *Strophanthus wightianus* Wall. ex Wight revealed the presence of therapeutically active compounds which possess antibacterial property. The antibacterial activity of cold water and ethanolic extracts of leaves of *Strophanthus wightianus* Wall. ex Wight was examined against 8 microorganisms and found to exhibit a promising antibacterial activity at 5 mg/ml against most of the studied Gram positive and Gram negative organisms. Table 1 indicates the results of the antimicrobial activities of the cold water extract of leaves of *Strophanthus wightianus* Wall. ex Wight with respect to the test organisms. The extract showed significant activity against eight test organisms. The data indicated that Gram-positive *Staphylococcus aureus* (MRSA) was the most sensitive strain of those tested with the cold water extract of leaves of *Strophanthus wightianus* Wall. ex Wight, with the strongest inhibition zone of 9.8 mm and the activity index of 0.613. The cold water extract also exhibited high antimicrobial to *Escherichia coli* (IZ-9.8 mm and AI-0.576), *Streptococcus mutans* (IZ-9.4 mm and AI-0.376), and *Escherichia coli* (IZ-10 mm and AI-0.588). The extract also showed moderate amount of antimicrobial activity against *Enterobacter aerogenes, Pseudomonas fluorescens, Klebsiella pneumoniae* and *Bacillus subtilis*. The inhibition zone observed for *Enterobacter aerogenes* was 8.2mm (AI-0.372),*Pseudomonas fluorescens* was 7.4 mm (AI-0.493), *Klebsiella pneumoniae* was 7.8 mm (AI-0.390) and for *Bacillus subtilis* was found to be 7.8 mm (AI-0.520). Table 2 depicts the results of the antimicrobial activities of the crude ethanolic extract of leaves of *Strophanthus wightianus* Wall. ex Wightwith respect to the selected test organisms. The extract showed noteworthy activity against 8 test organisms. The data indicated that *Enterobacter aerogenes* was the most sensitive strain of those tested with the ethanolic extract of leaves of *Strophanthus wightianus* Wall. ex Wight, with the strongest inhibition zone of 10.8 mm and the activity index of 0.675. The ethanolic extract also exhibited moderate antimicrobial activity against Methicillin resistant *Staphylococcus aureus* (MRSA) (IZ-8.8 mm and AI-0.440), *Klebsiella pneumoniae* (IZ-8.8 mm and AI-0.450), *Escherichia coli* (IZ-8.6 mm and AI-0.352), *Bacillus subtilis* (IZ-8.6and AI-0.505), *Pseudomonas fluorescens* (IZ-8.4 and AI-0.381), *Staphylococcus aureus*(IZ-8.4 and AI-0.560)and S*treptococcus mutans*(IZ-7.8 mm and AI-0.520). The inhibitory activity of these extracts confirmed the potential use of the plant in the treatment of microbial induced ailments. The ethanol extract was subjected to

different qualitative phytochemical tests for detection of different biologically active chemical groups. The results are summarized in Table 3. In these screening process alkaloides, anthraquinones, lipids/fats, glycosides, phenols, carbohydrates, tannins, resins, reducing sugars, saponins and flavanoids gave positive results while pholabtannins, terpenoids, sterols and acidic compounds gave negative results. The antibacterial activity found in the plant extracts have been attributed to some of the secondary metabolites [4, 5]. The presences of alkaloids and phenolic compounds are thought to be toxic to microorganisms, inhibiting the enzymes which are essential for the growth of microorganisms[6]. Modern medicines depend heavily on antibiotics for bacterial infections. However, the high genetic adaptability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance [7]. Thus there has been a continuing search for new and more potent antibiotics. According to World Health Report on infectious diseases 2000, overcoming antibiotic resistance is the major issue of the WHO for the next millennium[8]. Hence, the last decade has witnessed an increase in the investigations on plants as a source of human disease management.

Table 1

Antibacterial activity of cold water extract of leaves of Strophanthus wightianus Wall. ex Wight

Test Microorganism	Inhibition zone of Standard Ampicilin in mm	Inhibition zone (IZ) in mm	Activity Index (AI)
Bacillus subtilis	15	7.8	0.520
Enterobacter aerogenes	22	8.2	0.372
Escherichia coli	17	9.8	0.576
Klebsiella pneumoniae	20	7.8	0.390
Methicillin-resistant *Staphylococcus aureus* (MRSA)	16	9.8	0.613
Pseudomonas fluorescens	15	7.4	0.493
Staphylococcus aureus	16	8.8	0.550
Streptococcus mutans	25	9.4	0.376

Table 2

Antibacterial activity of ethanolic extract of leaves of Strophanthus wightianus Wall.ex Wight

Test Microorganism	Inhibition zone of Standard Ampicilin in mm.	Inhibition zone (IZ) in mm	Activity Index (AI)
Bacillus subtilis	17	8.6	0.505
Enterobacter aerogenes	16	10.8	0.675
Escherichia coli	16	8.6	0.375
Klebsiella pneumoniae	25	8.8	0.352
Methicillin-resistant *Staphylococcus aureus* (MRSA)	20	8.8	0.440
Pseudomonas fluorescens	22	8.4	0.381
Staphylococcus aureus	15	8.4	0.560
Streptococcus mutans	15	7.8	0.520

Table 3
Phytochemical screening of ethanolic leaf extract of Strophanthus wightianus Wall.ex Wight

No	Phytochemical components	Status
	Acidic compounds	-
	Alkaloids	+
	Anthraquinones	+
	Carbohydrates	+
	Flavanoids	+
	Glycosides	+
	Lipids/fats	+
	Phenols	+
	Pholabatannins	-
.	Reducing sugars	+
.	Resins	+
.	Saponins	+
.	Sterols	-
.	Tannins	+
.	Terpenoids	-

+ = positive; - =negative

The present study was conducted to extend newer lead for enhanced and safer chemotherapeutic means of the plant origin from endangered taxa of Western Ghats because these RET plant species keeps unrevealed with its potential uses. From the work results, it is observed that the ethanolic and cold water crude extracts of leaf of *Strophanthus wightianus* Wall. ex Wight showed antibacterial activity against most of the tested organisms especially *Staphylococcus aureus*. This indicates the potential of a new drug that has medicinal value as well as commercial viability.

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