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Research Article

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Assessment of Anti-bacterial Efficacy of Different Parts of the Medicinal Plant Wattakaka Volubilis (L.F.) Stapf Grown in Sri lanka

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Abstract Background: Plants have been recognized as a promising natural source for the discovery of novel drugs, including antibacterial agents. The present study evaluated *in vitro* antibacterial activity of aqueous and methanolic extracts of different parts of *Wattakaka volubilis* against two pathogenic bacterial strains, *Escherichia coli* (American Type Culture Collection 25922) *and Staphylococcus aureus* (American Type Culture Collection 25923). Agar well diffusion method was performed to evaluate the zone of inhibition while macro-broth dilution method was performed to determine minimum inhibitory concentration (MIC) of each test extract.

Results: Among the extracts tested, 500 mg/ml concentration of methanolic flower extract exhibited the maximum zone of inhibition against *E. coli* as well as *S. aureus*. Among the tested extracts the lowest MIC value was observed for methanolic flower extract (250 mg/ml) against *E. coli* as well as *S. aureus* (125 mg/ml). The maximum concentration of all the other tested extracts (500 mg/ml) also showed a significant ($p < 0.05$) anti-bacterial effect against both strains. Phytochemical analysis of flower extracts indicated the presence of carbohydrates, glycosides, flavonoids, diterpenes and alkaloids in both methanolic and aqueous extracts of flower. Only the aqueous extract contained Saponins.

Conclusion: The present study indicates that the different parts of the plant of the *W. volubilis* grown in Sri Lanka possess significant anti-bacterial effect against *E. coli* and *S. aureus.* Among them the flowers exhibited a higher activity than other parts of the plant.

Keywords Anti-bacterial activity, Minimum Inhibitory Concentration, *Wattakaka volubilis*

1. Introduction

Eradication of bacterial infections by bacteriostatic or bactericidal effects is achieved by using antibiotics [1-3]. Antibiotics act by either killing bacteria or by controlling their growth. Improper usage of antibiotics has been led to emergence of antibiotic resistant pathogenic strains and it has become a major health burden all over the world [4].

Sometimes excessive antibiotic intake may develop an allergic reactions, hypersensitivity and immunosuppression in patients [5]. Furthermore, when antibiotics do not respond and control the illness, it may become more complicated which leads to use of stronger and more expensive drugs. Thus the increase in emergence of antibiotic resistant bacteria increased the demand for alternative antibiotics from natural sources which are comparatively cheap with less adverse effects.

Sri Lanka possessed a rich culture with traditional medicinal system since 3000 years ago, which is mainly based on natural sources. The therapeutic value of many herbs using in traditional medicinal systems have been validated through scientific research including the anti-microbial activity [6]. Approximately 1414 plant species have been used in traditional medicinal system in Sri Lanka. Among them *Wattakaka volubilis* is a popular plant used in folk medicine, especially to treat wounds.

W. volubilis belongs to the family of Apocynaceae (Sub-family of Asclepidaceae). It is native to Sri Lanka and also found in India and West Bengal, Java and Indo-malysia. It is commonly called as "Sneeze wort". The plant is a tall woody climber densely lenticellate and pustular branches. Leaves are opposite and leaf blade is 5-13cm long and 3-10 wide, obtuse, truncate or shallowly cordate at the base and acuminate at the apex. Peduncle is longer than or same length of adjoining petioles [7]. The flowers are clusters, light green or yellowish green in colour, shine texture and consist of five petals. It is mildly scented but excluded in the evening and at night. The fruits are long, slender, and acuminate at the apex and applanate at the base. It is green in colour and is born as twins. At maturity the fruit split and seeds having silky, soft tufts fly quite far with the wind like parachutes [3].

The leaves of the *W. volubilis* are used traditionally in the treatment of fissures in the feet and rheumatic pain. The leaves are applied to treat boils and abscesses and it is also reported as a mild central nervous system depressant, anti-helminthic and antispasmodic. The roots of the plant reported to possess antipyretic activity and used in the treatment of kidney stones [8]. Tender stalks are used as an emetic and expectorant agent [1]. When the leaf powder taken orally along with cow's milk it shows the anti-diabetic activity [9].

A limited number of studies have been done to validate its usage as an antibacterial agent. There is no previous studies done on the antibacterial activity of this plant in Sri Lanka. Therefore the present study was designed to evaluate antibacterial activity of different parts of the plant *W. volubilis* grown in Sri Lanka.

2. Methodology

2.1. Sample collection and identification

The plant materials (Leaves, Roots, Stem and Flowers) were collected from Embilipitiya, Boralasgamuwa and Thelijjawila areas of Sri Lanka and pooled together. The Botanical identity of the plant was authenticated by National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka.

2.2. Preparation of plant extracts

The collected plant materials were washed with running water to remove contaminants. They were dried in open air under shade until a constant weight was achieved. Thereafter the materials were blended into a fine powder. The powdered plant materials (10 g) were suspended in 80 ml of distilled water and 99.8 % methanol in separate closed bottles. These were kept for 7 days with occasional shaking. "The extracts were filtered through Whatman No.1 filter paper into sterile glass bottles. Then the extracts were concentrated and stored under 4 ºC until further use".

2.3. Test micro-organisms and preparation of inoculums

Pathogenic strains of *Escherichia coli* (American Type Culture Collection 25922) and *Staphylococcus aureus* (American Type Culture Collection 25923) were obtained from Medical Research Institute, Colombo 08, Sri Lanka. These were cultured on nutrient agar plates and maintained on 2-4 ºC to be used for further studies. Around 3-4 bacterial colonies from each culture plate were added separately in to saline to prepare the bacterial suspensions equivalent to 0.5 McFarland standard.

2.4 Agar well diffusion Method

Bacterial suspensions (50 µl) were added on to agar plates and spread evenly on the agar using a sterile glass spreader. Four wells were cut in each agar plate and they were filled with 100 µl of 500 mg/ml, 250 mg/ml of each plant extract, positive control (Gentamycin 1 µg/100µl) and respective solvent as the negative control separately. Each test was repeated three times [10]. Then plates were kept for 2 hours at room temperature and then incubated at 37º C for 18-24 hours. After 24 h, the zone of inhibition of microbial growth was measured using a vernier caliper.

2.5. Measurement of Minimum Inhibitory Concentration

Macro-broth dilution method was performed to determine the minimum inhibitory concentration of each test extract. The bacterial suspensions of *E. coli* and *S. aureus* were added into nutrient agar broth separately. A series of dilutions for the concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml was prepared for each extract. The 1 ml of each microbial broth was then added separately to the tubes containing 1 ml of each dilution of each extract. Tubes were then incubated at 37ºC for 24 hours. This procedure was performed three times. Upon incubation, tubes were subjected for the determination of minimum inhibitory concentration (MIC). The lowest concentration of each extract which inhibit visible growth of micro-organisms was noted as MIC. To confirm the results, the absorbance of each sample was measured using VIS spectrophotometer (CT-2300 by ChromTech) at 625 nm.

2.6. 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 $P<0.05$.

2.7. Phytochemical Screening

The presence of different photochemical was determined in methanol and aqueous extracts of flowers of *Wattakaka volubilis.*

3. Results

3.1. *In-vitro* anti-bacterial activity against *E. coli*

Among the extracts tested, 500 mg/ml concentration of methanolic flower extract showed the largest zone of inhibition (Table 1) for *E. coli.* Both tested concentrations of methanolic flower extract (500 and 250 mg/ml) showed a significant inhibition compared to negative control ($P < 0.05$). However, aqueous flower extracts did not show a significant inhibition compared to negative control ($P > 0.05$).

The 500 mg/ml concentration of methanolic extract of root exhibited second largest zone of inhibition for *E. coli* (Table 2) compared to negative control ($P < 0.05$). The 250 mg/ml concentration of methanolic root extract also showed a significant inhibition compared to negative control ($P < 0.05$). The aqueous root extract of 500 mg/ml concentration showed the third highest zone of inhibition against *E. coli* and 250 mg/ml concentration of aqueous root extract also showed a significant inhibition compared to negative control ($P < 0.05$).

Both tested concentrations of methanolic leaf extract also showed a significant inhibition ($P < 0.05$) against *E. coli* compared to the negative control (Table 3). Nevertheless, the zone of inhibition for 500 mg/ml concentration was less than that of the aqueous root extract (500 mg/ml). The zone of inhibition showed by 500 mg/ml concentration of aqueous leaf extract ($P < 0.05$) was less than that of methanolic leaf extract (500 mg/ml). But 250 mg/ml concentration of aqueous leaf extract did not show a significant inhibition compared to the negative control ($P > 0.05$).

Although the anti-bacterial activity was less than the above said extracts, zone of inhibition against *E. coli* showed by (500 mg/ml, 250 mg/ml) methanolic stem extract was also significant compared to the negative control ($P < 0.05$). Among the extracts tested, 500 mg/ml concentration of aqueous stem extract exhibited the least zone of inhibition (Table 4) against *E. coli* as compared with the negative control ($P < 0.05$), while 250 mg/ml concentration of aqueous stem extract did not show a significant inhibition compared to the negative control ($P > 0.05$).

Table 1: Diameter of zone of inhibition for flower extracts of *W. volubilis* against *E. coli*

	Diameter of zone of inhibition in mm (Mean ± SEM)			
Extract	500 mg/ml	250 mg/ml	Positive	Negative
Aqueous	8.25 ± 0.53^{a}	5.005 ± 0.004^{a}	19.50 ± 0.707	5.00 ± 0.01
Methanol	$11.2 \pm 0.141^{*/a}$	$8.55 \pm 0.035^{*/a}$	18.20 ± 0.141	5.00 ± 0.02

Significance compared to negative control * $P < 0.05$, Significance compared to positive control ^{a} $P < 0.05$

Table 2: Diameter of zone of inhibition for root extracts of the root *W. volubilis* against *E. coli*

	Diameter of zone of inhibition in mm (Mean ± SEM)			
Extract	500 mg/ml	250 mg/ml	Positive	Negative
Aqueous	10.25 ± 0.176*/a	7.75 ± 0.093*/a	18.00 ± 0.00	5.00 ± 0.01
Methanol	10.50 ± 0.353*/a	7.40 ± 0.07*/a	19.00 ± 0.00	5.00 ± 0.01

Significance compared to negative control * P < 0.05, Significance compared to positive control [a] P < 0.05

Table 3: Diameter of zone of inhibition for leaf extracts of *W. volubilis* against *E. coli*

	Diameter of zone of inhibition in mm (Mean ± SEM)			
Extract	500 mg/ml	250 mg/ml	Positive	Negative
Aqueous	9.75 ± 0.53*/a	7.50 ± 0.353/a	18.00 ± 0.70	5.00 ± 0.02
Methanol	10.00 ± 0.004*/a	7.25 ± 0.176*/a	18.75 ± 0.105	5.00 ± 0.03

Significance compared to negative control * P < 0.05, Significance compared to positive control [a] P < 0.05

Table 4: Diameter of zone of inhibition for stem extract of *W. volubilis* against *E. coli*

	Diameter of zone of inhibition in mm (Mean ± SEM)			
Extract	500 mg/ml	250 mg/ml	Positive	Negative
Aqueous	7.50 ± 0.004*/a	6.20 ± 0.141/a	19.15 ± 0.106	5.00 ± 0.01
Methanol	9.25 ± 0.035*/a	8.10 ± 0.07*/a	19.95 ± 0.388	5.00 ± 0.03

Significance compared to negative control * P < 0.05, Significance compared to positive control [a] P < 0.05

3.2. *In-vitro* anti-bacterial activity against *S. aureus*

Among the extracts used, 500 mg/ml concentration of methanolic flower extract expressed the largest zone of inhibition (Table 5) against *S. aureus* compared to the negative control (P < 0.05). Also 250 mg/ml concentration of methanolic flower extract showed a significant inhibition compared with negative control (P < 0.05).

The second highest zone of inhibition was expressed by 500 mg/ml concentration of aqueous flower extract (Table 5) compared to the negative control (P < 0.05). The 250 mg/ml concentration of aqueous flower extract also showed a significant inhibition compared with the negative control (P < 0.05).

The third highest zone of inhibition was exhibited by 500mg/ml of methanolic extract of leaf (Table 6) compared with the negative control (P < 0.05). The 250mg/ml concentration of methanolic leaf extract showed a significant inhibition compared with the negative control (P < 0.05).

Although the anti-bacterial activity was less than the flower extracts and methanolic leaf extract, both tested concentrations (500 and 250 mg/ml) of methanolic stem extract exhibited a significant (P < 0.05) inhibition against *S. aureus* compared with the negative control (Table 7).

The 500 mg/ml concentration of aqueous leaf extract showed a lesser zone of inhibition against *S. aureus* than Methanolic leaf extract. But both tested concentrations (500 and 250 mg/ml) showed a significant (P < 0.05) inhibition compared with the negative control (Table 6).

The zone of inhibition against *S. aureus* showed by 500 mg/ml concentration of methanolic root extract (P < 0.05) was lesser than that of aqueous leaf extract (500 mg/ml), but higher than that of aqueous root extract (500 mg/ml). The 250 mg/ml concentration of methanolic root extract showed a significant inhibition compared with the negative control (P < 0.05), but same concentration of aqueous root extract did not show a significant (P > 0.05) inhibition compared with the negative control (Table 8).

The least zone of inhibition against *S. aureus* was exhibited by 500 mg/ml concentration aqueous stem extract ($P < 0.05$). Both tested concentrations (500 and 250 mg/ml) showed a significant ($P < 0.05$) inhibition compared with the negative control (Table 7).

Table 5: Diameter of zone of inhibition for flower extracts of *W. volubilis* against *S. aureus*

	Diameter of zone of inhibition in mm (Mean ± SEM)			
Extract	500 mg/ml	250 mg/ml	Positive	Negative
Aqueous	$10.15 \pm 0.035^{/a}$	$6.75 \pm 0.176^{/a}$	18.00 ± 0.00	5.00 ± 0.01
Methanol	$12.15 \pm 0.106^{*/a}$	$9.005 \pm 0.004^{*/a}$	18.00 ± 0.00	5.00 ± 0.03

Significance compared to negative control * $P < 0.05$, Significance compared to positive control ^{a} $P < 0.05$

Table 6: Diameter of zone of inhibition for leaf extracts of *W. volubilis* against *S. aureus*

	Diameter of zone of inhibition in mm (Mean ± SEM)			
Extract	500 mg/ml	250 mg/ml	Positive	Negative
Aqueous	$8.75 \pm 0.176^{*/a}$	$7.05 \pm 0.176^{*/a}$	19.5 ± 0.353	5.00 ± 0.02
Methanol	$9.2 \pm 0.14^{*/a}$	$7.6 \pm 0.07^{*/a}$	19.15 ± 0.212	5.00 ± 0.01

Significance compared to negative control * $P < 0.05$, Significance compared to positive control ^{a} $P < 0.05$

Table 7: Diameter of zone of inhibition for stem extracts of *W. volubilis* against *S. aureus*

	Diameter of zone of inhibition in mm (Mean ± SEM)			
Extract	500 mg/ml	250 mg/ml	Positive	Negative
Aqueous	$6.85 \pm 0.106^{*/a}$	$7.005 \pm 0.004^{*/a}$	18.00 ± 0.00	5.00 ± 0.02
Methanol	$9.10 \pm 0.07^{*/a}$	$8.15 \pm 0.106^{*/a}$	19.2 ± 0.141	5.00 ± 0.03

Significance compared to negative control * $P < 0.05$, Significance compared to positive control ^{a} $P < 0.05$

Table 8: Diameter of zone of inhibition for root extracts of *W. volubilis* against *S. aureus*

	Diameter of zone of inhibition in mm (Mean ± SEM)			
Extract	500 mg/ml	250 mg/ml	Positive	Negative
Aqueous	$7.15 \pm 0.106^{*/a}$	$5.02 \pm 0.007^{/a}$	16.00 ± 0.00	5.00 ± 0.01
Methanol	$8.5 \pm 0.35^{*/a}$	$7.00 \pm 0.01^{*/a}$	19.5 ± 0.353	5.00 ± 0.02

Significance compared to negative control * $P < 0.05$, Significance compared to positive control ^{a} $P < 0.05$

3.3. Minimum Inhibitory Concentrations for *E. coli* and *S. aureus*

The MIC values for different test extracts are presented in Table 9. Among the tested extracts the lowest MIC value was observed for methanolic flower extract (250 mg/ml) against *E. coli* as well as against *S. aureus* (125 mg/ml).

Table 9: Minimum Inhibitory Concentration for different extracts of *W. volubilis* against *E. coli* and *S. aureus*

Extract		**MIC for *E. coli***	**MIC of *S. aureus***
Flower	Methanolic	250 mg/ml	125 mg/ml
	Aqueous	500 mg/ml	125 mg/ml
Leaf	Methanolic	250 mg/ml	250 mg/ml
	Aqueous	250 mg/ml	500 mg/ml
Stem	Methanolic	500 mg/ml	500 mg/ml
	Aqueous	500 mg/ml	500 mg/ml
Root	Methanolic	500 mg/ml	500 mg/ml
	Aqueous	500 mg/ml	500 mg/ml

3.4. The phytochemical analysis of flower extracts of the *W. volubilis*

Phytochemical analysis of flower extracts indicated the presence of carbohydrates, glycosides, flavonoids, diterpenes and alkaloids in both methanolic and aqueous extracts of flower. Only the aqueous extract contained saponins.

4. Discussion

There is a timely need for discovery of new, more effective therapeutic agents to treat and control many diseases including bacterial infections. Due to presence with less adverse effects and low cost natural products are recognized as reliable sources for novel drug discovery.

The present study was designed to investigate the antibacterial activity of different extracts of the medicinal plant *W. volubilis.* It is evident from the results of the present study, that both methanol and aqueous extracts of the various parts of *W. volubilis* show an appreciable antibacterial activity against gram negative as well as gram positive bacterial pathogens. The maximum inhibition was shown by methanolic flower extract. The anti-bacterial effect of the flower extracts of *W. volubilis* evaluated for the first time and interestingly it exhibited a potent antibacterial activity than the other parts of the plant.

Furthermore, methanolic and aqueous extracts of root, leaf and stem also showed potential anti-bacterial activity against *E. coli* and *S. aureus*. Several other research groups also observed a significant antibacterial effect in different extracts of *W. volubilis* against many pathogenic bacteria. Thomas *et al.,* [11], documented *in vitro* antibacterial activity of methanol and acetone leaf extracts against gram positive and gram negative bacterial pathogens. A study done by Shibu and Dhanan [12] also observed the extracts from root, stem and leaves of *W. volubilis* exert anti-bacterial activity against gram positive and gram negative human pathogenic bacteria. Venkatesan *et al.,* [13] revealed leaf extract showed antibiotic activity against *Bacillus pumilus, E. coli* and *Pseudomonas aeruginosa*. Yogita *et al*., [1] reported that the ethanolic extract of *W. volubilis* possesses potential antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa, Klebsiella species.* The results of the present study confirms the observations of previous studies done on anti-microbial activity of different parts of the *W. volubilis* grown in other countries.

In general, the results indicated that the methanolic extracts expressed higher anti-bacterial activity than aqueous extracts against both bacterial strains. The effectiveness of the extracts largely depends on the type of solvent used, because the phytochemical profile of an extract determines by the polarity of the compounds being extracted in a given solvent. The most antibacterial active components in plant materials are saturated organic molecules [14]. Hence, active lipophilic constituents do not extract in to aqueous extract. Therefore organic extracts exhibit more potent antibacterial activity as compared to aqueous extracts. Preethi *et al* [15] showed that the alcoholic extract of *Leucas aspera* and *Holarrhena antidysenterica* exhibit more powerful antimicrobial effect compare to aqueous extract. Another study done by Akinsulire *et al*., [16] observed that the gram-positive organisms were more sensitive to the methanol extract of *Bryophyllum pinnatum*, while the extracts from other solvents including aqueous extract showed moderate to weak activity. Thus it is evident that organic extracts exhibit more potent antibacterial activity as compared to aqueous extracts.

The results of the present study revealed that the majority of test extract exhibited comparatively higher antibacterial activity against *E. coli* than *S. aureus.* The reason for this observation may be due to the chemical and structural difference in the cell walls of gram positive and gram negative bacteria. Gram positive bacteria contain thick peptidoglycan cell wall along with teichoic acid but gram negative bacterial contain only thin peptidoglycan cell wall [17]. Therefore, the active compound present in test extract may reach the gram negative bacterial cell more readily, whereas they have to penetrate through the thick cell wall to reach the bacterial cell in gram positive strains. The antibacterial compounds in test extracts may reach the *E. coli* bacterial cells more efficiently than *S. aureus* bacterial cells and exert their antimicrobial effect since *E. coli* is a gram negative bacterial while *S. aureus* is a gram positive bacteria.

The observed MIC values, confirmed the antibacterial activity exhibited by the zone of inhibition by each test extracts. A study done by Yogita *et al* [1] reported that MIC values ranged from 500-1000 µg/ml for the ethanolic root extract of *W. volubilis.* A study done on the squeezed-leaf juice of *Kalanchoe crenata* observed MIC values of 64 mg/ml against *E. coli* and 128 mg/ml against *S. aureus* [16].

In the present study, phytochemical screening was performed for the flower extracts, which showed the maximum antibacterial activity. The results indicated the presence of carbohydrates, glycosides, flavonoids, diterpenes and alkaloids in both methanolic and aqueous extracts, while only the aqueous extract contained saponins. This is the first attempt to investigate the phytochemicals present in the flower extracts. Venkatesan *et al,* [13] reported that alkaloids, anthroquione, caumarin, flavanoids, phenols, saponins, steroids, tannins and

terpenoids are present in ethanol, methanol, petroleum ether and DMSO extract from the leaf of *W. volubilis.* Another study done on ethanol extract of leaves was found to have saponins, coumarins and phytosterols [18]. Bharathamma and Sudarsanam [19] revealed the presence of Alkaloids, Terpenoids, Steroids, Coumarins, Tannins, Flavonoids, Proteins, Carbohydrates, Glycosides, Phytosterol, Anthocyanidins, Amino acids and Lipids in the aqueous fruit extract of *W. volubilis.* Thus the results of the present investigation get confirmed by the observations of previous studies.

5. Conclusion

The results of the present investigation confirm the anti-microbial potency of leaves, stem, and roots of the plant of *W. volubilis*. In addition, it revealed that the flower extracts of the plant possess a potent anti-bacterial activity. Hence, the therapeutic value of *W. volubilis* as a medicinal plant which is widely used in traditional medicinal systems is scientifically validated via the observations of the present study.

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