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A Comparative Study on *In Vitro* Antibacterial Activity of Different Leaf Extracts of Medicinal Plant *Talinum paniculatum*

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ABSTRACT

There is an urgent need for the development of novel affordable plant based natural antibacterial agents in order to combat against the emergence of antibiotic resistance, resulting from the indiscriminate use of currently available antibiotics. Therefore, this study evaluated the *in vitro* antibacterial activity and minimum inhibitory concentrations (MIC) of aqueous, methanol, acetone and hexane extracts of the leaf *Talinum paniculatum* against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923). Agar well diffusion method was performed to evaluate antibacterial activity of each leaf extract at concentrations of 100 mg/mL and 50 mg/mL. Additionally, broth dilution technique was used to determine MIC. The results revealed, for the first time, that each crude leaf extract exhibited antibacterial activity against both *E. coli* and *S. aureus*. The largest zones of inhibition against *E. coli* and *S. aureus* were observed for 100 mg/mL methanolic and aqueous leaf extracts, respectively. It is concluded that antibacterial potential of the leaf *T. paniculatum* is mediated primarily via its phytoconstituents, such as alkaloids, phenols, saponins, tannins, steroids, triterpenes and flavonoids. These bioactive constituents present in the leaf are able to hinder peptidoglycan synthesis in bacterial cell wall, destroy bacterial membrane structures, alter surface hydrophobicity, change cell signaling pathways and modulate quorum-sensing and therefore, restrict the growth of microorganisms. Consequently, leaf of *T. paniculatum* is a promising source to develop potent, cheap and natural antibacterial agents.

1. INTRODUCTION

Antimicrobial resistance has become a serious public health burden at present. Misuse and overuse of antimicrobial agents, particularly antibiotics, accelerate the process of antimicrobial resistance as it allows microorganisms, such as bacteria, to alter their genetic make-up in order to withstand the effect of antimicrobial agents [1]. Rapid rise of antibiotic resistance risks the potential to treat common infectious diseases and leads to prolonged illness, disability and death worldwide [1,2]. As a result, infections caused by antibiotic-resistant bacteria are often difficult to treat and demand alternative drugs or higher doses, inducing some serious side effects. Additionally, healthcare expenditure required to cure resistant infections is usually greater than that of non-resistant infections [1,3,4]. Consequently, there is a necessity and great demand for development of novel efficacious plant based natural antibacterial drugs which are relatively cheap with less adverse effects.

In this context, researchers have focused on antibacterial potential of various herbal plant extracts used in Ayurveda, traditional and folk medicine. Among herbs, *Talinum paniculatum* is widely used in many countries across the globe for various medical conditions due to its promising medicinal and healing properties [5,6]. *T. paniculatum* belongs to the family Portulacaceae, and it is native to tropical America [5,7]. However, *T. paniculatum* is a pantropical plant found in several countries including Brazil, Ghana, Nigeria, Mexico and Sri Lanka [5,7,8]. It is a perennial subshrub, and it can reach up to 30 cm in height. Morphologically its leaves are fleshy, juicy, petiolate and oppositely arranged on the stem with smooth-edged. Further, leaves and young stem of the plant is edible and use as a vegetable for human consumption. It has small pink colour flowers with 4-6 petals. *T. paniculatum* is also known as “Jewels of Opar” or “Fameflower” in common [5,6,7,8].

In traditional medicine, leaves and roots of *T. paniculatum* have been used to treat wide range of diseases including diarrhoea, dysmenorrhoea, lung diseases, spleen disorders, impotence, male infertility, gastrointestinal disorders and conditions of urine with bad smelling. Besides, this plant is also used to treat ulcers, wounds and skin infections in folk medicine [5,6,7]. Phytochemically, leaves are very rich in flavonoids, saponins, tannins, steroids and triterpenes [5,9]. Most importantly, anti-diuretic, anti-diarrhoeal, anti-inflammatory, aphrodisiac, anti-nociceptive and edematogenic activities of the leaf *T. paniculatum* are scientifically proven and well-documented [6,7,9]. Nevertheless, existing scientific knowledge on its antibacterial activity is limited and not well-proven, especially in the

context of Asia, though its leaves and roots are extensively used to treat some bacterial induced infections in many South-East Asian countries including Sri Lanka [10].

This study was conducted to determine *in vitro* antibacterial activity of aqueous, methanol, acetone and hexane extracts of the leaf *T. paniculatum* against *Escherichia coli* and *Staphylococcus aureus*. To the best of present knowledge, this is the first study that reports antibacterial potential of aqueous, methanol, acetone and hexane crude extracts of the leaf *T. paniculatum*.

2. MATERIALS AND METHODS

2.1. Collection of plant material

Fresh matured leaves of the plant *T. paniculatum* were collected from Kurunegala district, Sri Lanka ($7^{o}29'12"N$ $80^{o}21'53"E$) in April 2017.

2.2. Identification of plant material

The plant material was identified by Prof. P. Tissera, Professor of Botany, Department of Botany, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka in comparison to the herbarium specimens of the Department. A voucher specimen (USJP FMS 7/2010) has been deposited at the herbarium of the Department of Botany, Faculty of Medical Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.

2.3. Extracts preparation

Fresh matured leaves of *T. paniculatum* were washed twice in running tap water. Leaves of *T. paniculatum* were then air dried for seven days until a constant weight was obtained. The dried leaves were powdered using a mortar and pestle. The powder (8.0 g each) was subjected to extraction with 100 mL of sterile distilled water, methanol, acetone and hexane separately for 7 days. Each extract was filtered through a Whatman filter paper. Filtrates were freeze dried and stored in sealed beakers. All filtrates were then kept in a refrigerator ($4^{o}C$) until use.

2.4. Test microorganisms

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

2.5. Preparation of inoculums

Two culture plates were prepared for *E. coli* and *S. aureus* from the above plants. Around 3-4 bacterial colonies from each culture plate were then added separately to the tubes containing 4 mL of saline and bacterial suspensions equivalent to 0.5 McFarland standard were prepared for both *E. coli* and *S. aureus*.

2.6. Agar well diffusion method

Agar well diffusion method was used to screen antibacterial activity. Nutrient agar plate surface was inoculated by spreading 200 µL of microbial suspension over the entire agar surface. Four wells with a diameter of 8 mm have punched aseptically around the plate parameter, at least 2.5 cm apart, with a sterile tip in each nutrient agar plate. Each agar plate was then introduced with 100 µL of two dilutions (100 mg/mL and 50 mg/mL) of each leaf extract separately, in addition to the positive and negative controls. The 1 µg/mL concentration of Gentamycin was used as a positive control, whereas distilled water, methanol, acetone or hexane was used as a negative control with respect to the extract used. This procedure was performed in triplicates for both microbial suspensions of *E. coli* and *S. aureus*. Agar plates were then incubated at 37^{0}C for 24 hours before the diameter of any zone of inhibition was noted and measured.

2.7. Determination of minimum inhibitory concentration (MIC)

Microbial broths were prepared by adding 1 mL of above-prepared bacterial suspensions of *E. coli* and *S. aureus* into 150 mL of nutrient agar broth (1:150 ratio) separately. Series of dilutions for the concentrations of 100 mg/mL, 50 mg/mL and 25 mg/mL were prepared for each leaf extract separately. The 1 mL of each microbial broth was then added separately to the tubes containing 1 mL of each dilution of each extract. This procedure was performed in triplicates. Tubes were then incubated at 37^{o}C for 24 hours. Upon incubation, tubes were subjected for the determination of minimum inhibitory concentration (MIC). The lowest

concentration of each extract that inhibits the visible growth of test microorganisms was designated as the MIC.

2.8. 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

3.1. *In vitro* antibacterial activity against *E. coli*

The results of *in vitro* antibacterial activity are depicted in Table 1 and 2. As shown in Table 1, among the extracts used, 100 mg/mL concentration of methanolic leaf extract showed the largest zone of inhibition for *E. coli* compared to the negative control ($P<0.01$). However, 50 mg/mL methanolic leaf extract did not show a significant inhibition compared to the negative control ($P>0.05$).

The 100 mg/mL concentration of aqueous and acetone extracts exhibited second largest zone of inhibition against *E. coli* compared to the respective negative controls ($P<0.01$). Additionally, 50 mg/mL concentration of aqueous leaf extract also showed a significant inhibition against *E. coli* ($P<0.01$), while the same concentration of acetone extract did not yield a significant zone of inhibition compared to the negative control ($P>0.05$).

Among the extracts tested, 100 mg/mL concentration of hexane extract exhibited the least zone of inhibition against *E. coli*. Yet, the antibacterial potential expressed by both 100 mg/mL and 50 mg/mL concentrations of hexane extracts were significant compared to the negative control ($P<0.01$).

3.2. *In vitro* antibacterial activity against *S. aureus*

As shown in Table 2, the largest zone of inhibition against *S. aureus* was expressed by the 100 mg/mL aqueous leaf extract and this effect was significant compared to the negative control ($P<0.01$). The 100 mg/mL concentration of methanol and acetone extracts also exhibited a significant inhibition against *S. aureus* with respect to their negative controls ($P<0.01$), even though the diameter of zones of inhibition were lesser than that of the aqueous

leaf extract. Besides, 100 mg/mL hexane leaf extract yielded the smallest zone of growth inhibition against *S. aureus*. Nevertheless, 100 mg/mL hexane leaf extract exhibited significant inhibition with respect to the negative control (P<0.01).

The 50 mg/mL concentration of aqueous and methanolic leaf extracts also showed a significant inhibition against *S. aureus* compared to the respective negative controls (P<0.01). However, 50 mg/mL acetone and hexane leaf extracts did not show a significant inhibition against *S. aureus* compared to respective negative control (P>0.05).

Gentamycin, positive control, showed the largest zones of inhibition against both *E. coli* (P<0.001) and *S. aureus* (P<0.001) compared to the negative controls used in this study. Further, any of the leaf extract at any concentration did not show a significant inhibition compared to Gentamycin (P>0.05) due to the appearance of comparatively small zones of inhibition. These results indicate 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.

Table 1: Antibacterial screening test results of different extracts of the leaf *T. paniculatum* against *E. coli*

Extract	Diameter of zone of inhibition in mm (Mean ± SEM)			
	100 mg/mL	50 mg/mL	Positive control	Negative control
Distilled water	14.67±0.33**/^{c}	11±0.00**/^{c}	35.00±0.58***	8.33±0.33
Methanol	16.00±0.58**/^{c}	8.33±0.33^{c}	36.00±0.58***	9.00±0.00
Acetone	14.67±0.33**/^{c}	8.33±0.33^{c}	34.00±0.58***	8.00±0.00
Hexane	11.33±0.33**/^{c}	11±0.00**/^{c}	33.00±0.58***	8.33±0.33

Significance compared to negative control P<0.05*, P<0.01**, P<0.001***

Significance compared to positive control P<0.05^{a}, P<0.01^{b}, P<0.001^{c}

Table 2: Antibacterial screening test results of different extracts of the leaf *T. paniculatum* against *S. aureus*

Extract	Diameter of zone of inhibition in mm (Mean ± SEM)			
	100 mg/mL	50 mg/mL	Positive control	Negative control
Distilled water	18.00±0.58**/ ^{b}	13.33±0.33**/ ^{c}	32.00±0.00***	8.67±0.33
Methanol	15.00±0.00**/ ^{b}	12.00±0.00**/ ^{b}	29.00±0.58***	8.67±0.33
Acetone	15.00±0.58**/ ^{b}	8.67±0.33^{c}	32.00±0.58***	8.00±0.00
Hexane	12.33±0.33**/ ^{b}	8.33±0.33^{c}	30.00±0.58***	8.00±0.00

Significance compared to negative control P<0.05*, P<0.01**, P<0.001***

Significance compared to positive control P<0.05^{a}, P<0.01^{b}, P<0.001^{c}

3.3. Analysis of MIC

Among the concentrations used in this study, for *E. coli*, MIC value of aqueous, methanol and acetone extracts of the leaf *T. paniculatum* was 100 mg/mL, whereas 50 mg/mL was the MIC of hexane leaf extract. Additionally, for *S. aureus*, aqueous and methanolic leaf extracts exhibited a MIC value of 50 mg/mL, while MIC of acetone and hexane extracts was 100 mg/mL.

4. DISCUSSION

Herbal plants have been used in traditional medicine since ancient times due to their curative properties [5]. WHO recognizes medicinal plants as the best source to obtain a wide range of drugs [11]. Plants are explored as possible sources of novel therapeutic agents in drug discovery at present [5,12]. In this connection, this study assessed *in vitro* antibacterial activity of aqueous, methanol, acetone and hexane extracts of the leaf *T. paniculatum*. The experimental condition and setup used in this study were similar to which has been used by several other investigators [13,14,15]. Further, *E. coli* and *S. aureus* were selected as test microorganisms due to their clinical significance.

Occurrence of antibiotic resistance in *E. coli* is of a particular concern given the fact that *E. coli* is the most common Gram-negative pathogen in humans and leading cause of urinary tract infection (UTI) [16,17]. In addition, *S. aureus*, a Gram-positive bacterium, increasingly

shows resistance to multiple antimicrobial agents [18], and Asia has been identified as the region with the highest incidence of methicillin-resistant *S. aureus* (MRSA) in the world [19].

Results of this study revealed that the aqueous, methanol, acetone and hexane crude extracts of the leaf *T. paniculatum* possesses *in vitro* antibacterial potential against *E. coli* and *S. aureus*, and this is a novel finding. 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 antibacterial [13].

Among the extracts tested, the highest antibacterial activity (exhibiting the largest zone of inhibition) was expressed by the aqueous leaf extract of *T. paniculatum* against *S. aureus* and its MIC value was 50 mg/mL. Moreover, methanol, acetone and hexane extracts also showed antibacterial potential against *S. aureus*. The anti-staphylococcal activity found in the leaf *T. paniculatum* is significant due to the emergence of MRSA strains, and they are the main causes of several hospital and community acquired infections [19,20]. Similarly, Reis *et al*. [2015] also reported *in vitro* antibacterial activity of hydroalcoholic leaf extract of *T. paniculatum* along with its hexane and ethyl acetate fractions against selected Gram-positive bacteria including *S. aureus*. Thus, antibacterial potential of the leaf *T. paniculatum* is also reported in previous research as evident in this study regardless of the type of leaf extract used.

This study also showed *in vitro* antibacterial activity of aqueous, methanol, acetone and hexane extracts of the leaf *T. paniculatum* against *E. coli*. Among the crude extracts, methanolic leaf extract expressed a noteworthy antibacterial activity against *E. coli* and its MIC value was 100 mg/mL. Even though this study reports *in vitro* antibacterial property of leaf *T. paniculatum* against *E. coli* for the first time, few previous studies have also reported *in vitro* antibacterial activity of plant extracts that are belonging to family Portulacaceae. For instance, antibacterial activity of the leaf *Portulaca oleracea* L. was noted against some Gram-negative strains, such as *E. coli* and *P. aeruginosa* [21].

Interestingly, crude leaf extracts of *T. paniculatum* exhibited comparatively high antibacterial activity against *S. aureus* compared to *E. coli*. This could be due to the chemical and structural differences in the cell walls of these bacteria. Gram-positive bacteria have less chemically and structurally complex cell walls than Gram-negative bacteria [22].

Consequently, it is considerably easy for the *T. paniculatum* leaf extracts to disrupt the cell wall of *S. aureus* and inhibit their growth to a larger extent as demonstrated in this study.

Study findings clearly indicate that the antibacterial effect observed for both *E. coli* and *S. aureus* is genuine and intrinsic. All the tested leaf extracts showed a significant activity compared to respective negative controls. However, this effect is not comparable to the reference drug for the tested concentrations. The higher concentrations of each extract may possess more potent antibacterial activity and therefore, there is a very high potential to develop efficacious and affordable antibacterial agent(s) from the leaf *T. paniculatum*.

The antibacterial property exhibited by the leaf *T. paniculatum* is attributed to various bioactive constituents present in the plant, including alkaloids, phenols, saponins, tannins, steroids, triterpenes and flavonoids [5,9,23]. The above compounds are commonly termed as "phytochemicals," and they are known as naturally occurring pharmacologically active compounds in plants [12]. It is well known that phytoconstituents found in herbal plants account for their versatile health benefits. For instance, phenols, organic compounds consisting hydroxyl group, are typically known for their health promoting properties, such as anti-oxidant, antibacterial and anti-inflammatory activities [12,24,25]. In pharmaceutical industry, triterpenes have been referred to as antibiotic, insecticide, anthelmintic and antiseptic [25]. Besides, researchers have investigated antibacterial potency of all of the above-mentioned biologically active compounds found in the leaf *T. paniculatum* [26,27,28,29,30,31,32].

Phytoconstituents, particularly secondary metabolites including phenolic compounds and tannins, are known to be synthesized by plants in response to various microbial induced diseases including bacterial infections [33,34]. Hence, they are found *in vitro* to be effective antibacterial agents against a spectrum of bacteria as evident in this study. Phytochemicals, such as phenols, saponins and tannins, are able to obstruct peptidoglycan synthesis in bacterial cell wall, destruct bacterial membrane structures, alter surface hydrophobicity of bacterial membrane and change the signal transduction [12,35]. Interestingly, phytochemicals are also capable of inhibiting or modulating quorum-sensing, a process that enables bacteria to restrict specific gene expression, in pathogenic bacterial strains [36,37]. Alkaloids possess antibacterial potential which is mediated via interactions with bacterial DNA [38]. Consequently, extracts of the leaf *T. paniculatum* tend to illustrate antibacterial potency

against *E. coli* and *S. aureus* via single or multiple above-mentioned mechanisms as evident in this study.

Predominantly, aqueous and methanolic extracts of the leaf *T. paniculatum* exhibited a promising antibacterial potency than acetone and hexane extracts. Previous studies have been identified that methanol, ethanol and water as suitable solvents to extract most of the bioactive compounds in plants [34]. This could be the reason for greater antibacterial activity showed by aqueous and methanolic leaf extracts in this study as these extracts are very rich in biologically active compounds that are responsible for antibacterial potential.

5. CONCLUSION

In conclusion, this study, for the first time, showed *in vitro* antibacterial potential of the leaf *T. paniculatum*, and it is primarily mediated via the bioactive compounds present in the leaf. There is a strong possibility exists to develop a novel, safe, cheap and natural antibacterial agent(s) from the leaf *T. paniculatum*. Future research focusing on high concentrations of different leaf extracts is recommended for better understand the antibacterial effect of the leaf *T. paniculatum*.

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