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In-vitro evaluation of antibacterial activity of *Tabebuia pallida* against multi-drug resistant bacteria

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Abstract
The present study was undertaken to explore the antimicrobial potential of different parts of *Tabebuia pallida*. The methanol crude extract of different parts (leaves, stem bark, root bark and flowers) of the plant were used for screening antimicrobial potentials using disk diffusion and serial dilution method. Among the tested pathogens *E. coli* was highly susceptible to all parts of *T. pallida* except root extracts. Compare to other extract the flower part was highly sensitive against all tested pathogens with maximum zone of inhibition 21 mm. The MIC value 125 µg/ml of FE against *E. coli* was least in comparison to other part extracts and MBC was also similar to MIC value. In conclusion, the spectrum of activity suggests that methanol extracts of *T. pallida* flower could be a possible source to get noble and potential herbal medicines to treat infections, hence justified the ethnic uses of this plant against various infectious diseases.

Keywords: Antimicrobial activity, *Tabebuia pallida,* multi-resistance, minimum inhibitory concentrations, infectious diseases

1. Introduction
In the last three decades, we have achieved enormous new antibiotics due to advances in technology and science to combat different type of infectious disease caused by pathogens [1]. However, these antibiotics have failed to fulfill our optimism to control life threatening infectious diseases due to rise in the transitional nature of infectious diseases. In general, bacteria which cause infectious diseases have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [2]. Such a fact is cause for concern, as infectious diseases which are associated with high morbidity and mortality are the leading cause of death world-wide. Antibiotic resistance has become an alarming issue globally due to exposure of new bacterial strains "Superbugs", which are multi-resistant [3-5]. Now a day's such multidrug resistance properties of a pathogen have threatened the clinical efficacy of many existing antibiotics [6].
Due to increase the resistance of antibiotics, there is a continuous and urgent need to discover new antimicrobial agents with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [7]. Among the potential sources of new agents, plants have long been investigated. Plants are rich in secondary plant metabolites (phytochemicals), such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinines, which have been used globally in traditional medicine to treat several infectious diseases [8-10]. It has been reported that natural products, either as pure compounds or as standardized plant extracts have multi-antimicrobial properties provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity [11, 12]. Therefore, researchers are increasingly turning their attention towards medicinal plants, looking for new leads to develop better drugs against microbial infections.
Tabebuia pallida (*T. pallida*, commonly known as White trumpet tree), belonging to the largest genera of the Bignoniaceae family distributed in central America, West India and South America [13, 14]. *Tabebuia* genus is commonly recognized as a therapeutic alternative by rural or remote populations in the treatment of different diseases. To our knowledge from literatures, there is no work about phytochemical contents, antimicrobial and biological activities of *T. pallida*. However, literature review on *Tabebuia* genus showed variety of biological activities [13]. Several Tabebuia species have been used in traditional medicine to treat infectious diseases. For example Lapachol first isolated from *Tabebuia avellanedae* has

antibacterial, antiviral, antiparasitic and antifungal activities [15, 16]. *T. rosea* is used as antipyretic, anti-inflammatory, antibacterial, antifungal, anti-cancer and anti-diabetic agents [14, 17-19]. Stem bark of *T. avellanedae* is used in the treatment of snake bite [14, 20]. Also, *T. heterophylla*, *T. aurea*, *T. argentea*, *T. caraiba* are used as anticancer, anti-inflammatory and antimicrobial agents [13, 21].

The extensive use of *Tabebuia* species for the treatment of infectious diseases in traditional medicine and identification of bioactive lapachol has motivated us to investigate the antimicrobial activity of *T. pallida* to validate or otherwise prove the claims of the herbalists who use it as an antimicrobial remedy. However, a study with focusing on antibacterial activity of different parts of *T. pallida* has not yet been documented. Previously we reported the free radical scavenging activity of different parts of this plant [22]. Therefore, the objective of this study was to evaluate the antibacterial activity of the methanol extract from different parts of *T. pallida* including leave, flower, stem and root against some most important human pathogenic bacteria.

2. Materials and Methods

2.1 Plant collection

Leaves, flowers, stem and root barks of *T. pallida* were collected from Rajshahi University Campus, Rajshahi, Bangladesh on May, 2013 and were identified by an expert taxonomist at the Department of Botany, University of Rajshahi, where a voucher specimen (Voucher No. MN-03) was deposited. Plant materials were then washed separately with fresh water to remove dirt and other contaminants, and were shade-dried for several days with occasional sun drying. The dried materials were ground into coarse powder by a grinding machine and the materials were stored at room temperature (RT) for future use.

2.2 Preparation of the extract

The extraction was performed according to Alam *et al*. [23]. About 500 g of each powdered plant materials was taken in four amber colored extraction bottles and soaked with 1.5 L of methanol. The sealed bottles were kept for 15 days with occasional shaking and stirring. The extracts were filtered separately through a fresh cotton plug and finally with Whatman No.1 filter papers. The filtrates were concentrated with a rotary evaporator (Bibby Sterlin Ltd, UK) under reduced pressure at 50° C to afford 30, 35, 45 and 40 g extract of leaves, flowers, stem and root barks extract, respectively.

2.3 Test Micro-organisms

Antimicrobial activity of different parts of *T. pallida* were determined against two Gram positive (*Staphylococcus aureus and Bacillus cereus*) and four Gram negative bacteria (*Escherichia coli, Shigella dysenteriae, Shigella sonnei and Agrobacterium species*). Test organisms were the laboratory stocks of Microbiology Lab, Pharmacy Department, Rajshahi University Bangladesh.

2.4 *In-vitro* antibacterial activity

In-vitro antibacterial activity was carried out on nutrient agar plate by disc diffusion method [24]. The crude extracts of different parts of *T. pallida* were separately dissolved in 1 ml of methanol solvent. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts (300µg/disc) of the test substances using micropipette. Discs were placed on agar plate culture of test organisms by sterilized forceps. The inoculated agar plates were left in refrigerator for one hour for proper diffusion. The plates were then allowed to incubate at 37° C for overnight. Standard antibiotic discs Kanamycin (KN) 10µg/disc and blank discs (impregnated with solvents) were used as a positive and negative control. The antibacterial activity of the test agents was determined by measuring the diameter of zone of inhibition, expressed in mm.

2.5 Determination of Minimum Inhibitory Concentration (MIC)

The plant parts that showed highest antimicrobial activity against respective bacterial strain were later tested to determine the MIC and MBC values. The minimum inhibitory concentrations (MIC) were performed by a serial dilution technique according to the NCCLS protocol [25]. The extracts were diluted to give the final concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.91 µg/ml. 10 µ l of 10^7cell/ml of the tested microorganism was inoculated in tubes with equal volume of nutrient broth and plant extracts. MIC was read in µg/ml after overnight incubation at 37 °C. Three control tubes were maintained for each strain (media control, organism control and extract control). The lowest concentration (highest dilution) of the extract that produced no visible growth (no turbidity) in comparison with control was defined as MICs.

2.6 Determination of Minimum Bactericidal Concentration (MBC)

MBC value was determined by sub culturing the test dilution (which showed no visible turbidity) on to freshly prepared nutrient agar media. The plates were incubated further for 18-42 h at 37° C. The highest dilution that yielded no single bacterial colony on the nutrient agar plates was taken as MBC. If growth of bacteria is observed in the MIC tubes, it indicated the presence of bacteriostatic agents and in this case MBC>MIC. No growth of bacteria in the tubes after dilution indicates the presence of bactericidal agent and in this case, MIC=MBC.

3. Results

In the present antimicrobial screening, the inhibitory effect of different parts (leaves, flowers, stem bark and root bark) of *T. pallida* methanol extracts were evaluated against both gram positive and gram negative bacterial strains. Table 1 and 2 summarizes the microbial growth inhibition of different part extracts of the experimental plant species. The activity was quantitatively assessed on the basis of inhibition zone and minimum inhibitory concentration (MIC) with their bactericidal efficiency (MBC).

3.1 Antibacterial activity

The antibacterial potential of plant extracts was evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standards; KN (10 µg /disc). The result shown that, among the four parts of *T. pallida* plant extracts tested by the disk diffusion method, only flower part was susceptible against all tested pathogenic bacteria. The methanol root extracts of *T. pallida* did not show activity against any of the tested bacterial strains (Table 1). The results obtained indicate that all the extracts except root bark extract tested were efficient against *E. coli* with zone of inhibition 12, 11 and 21mm by LE, SBE and FE respectively where the standard kanamycin showed 39 mm diameter of zone inhibition. In case of gram positive bacteria *Staphylococcus aureus and*

Bacillus cereus only LE and FE shown antibacterial susceptibility 10, 15 and 9, 11mm zone of inhibition respectively. On the other hand among the gram negative bacteria maximum inhibition 21 mm was observed by FE against *E. coli* in comparison to other sensitive parts of *T. pallida*. *Shigella dysenteriae* and *Shigella sonnei* were susceptible only by FE with 13 and 9 mm diameter of zone inhibition respectively, while other parts did not show any activity. Moreover, *Agrobacterium species* was susceptible by FE 11mm and LE 8mm respectively while SBE and RBE were non active. Though, the FE was highly sensitive against all tested bacterial strains but the maximum zone of inhibition 21mm against *E. coli* was moderately considerable with the standard kanamycin 39 mm.

Table 1: Antibacterial activity (zone of inhibition, mm) of various parts of *T. pallida* against gram positive and gram negative bacteria.

Types of organism	Name of organisms	Zone of inhibition in mm at 300 µg/disc				
		LE	SBE	FE	RBE	KN
Gram positive Bacteria	*Bacillus cereus*	10	NA	15	NA	34
	Staphylococcus aureus	9	NA	11	NA	37
Gram negative Bacteria	*Escherichia coli*	12	11	21	NA	39
	Shigella dysenteriae	NA	NA	13	NA	34
	Shigella sonnei	NA	NA	9	NA	32
	Agrobacterium species	8	NA	11	NA	34

LE = Leaves extract, SBE = Stem bark extract, RBE= Root bark extract, FE = Fruits extract, KN = Kanamycin, NA = No activity.

3.2 Determination of MIC and MBC values

The MIC and MBC values obtained for the methanol extracts of *T. pallida* against highly susceptible pathogenic microorganism *E. coli* supports their antibacterial efficacy as like their zone of inhibition activity. The results shown in Table 2 reported that methanol extract of different parts except root part of *T. pallida* posses considerable antimicrobial agent. Among different parts of *T. pallida* the FE showed lowest MIC value 150 µg/ml where as SBE and LE showed same MIC value 250 µg/ml against most susceptible micro-organism *E. coli*. The minimum bactericidal effect was obtained by FE, where MIC and MBC value were equal (150 µg/ml) suggesting that the plant extracts were bactericidal in nature against respective organism. The MBC value of SBE and LE was higher than their MIC value represents that the plant extract were bacteriostatic at lower concentration and bactericidal at higher concentration against tested pathogen *E. coli*.

Table 2: MIC (µg/ml) and MBC performance of different parts *of T. pallida* against *E. coli.*

Name of samples	MIC	MBC
LE	250	>250
SBE	250	>250
FE	125	125

4. Discussion

Antibiotics are the basis for the therapy of microbial infections. However, antibiotic resistance due to high genetic variability of microbes has appeared as the issue of global concern. According to World Health Report of Infectious diseases 2000, antibiotic resistance overcoming is the major issue of the WHO for the next millennium. Thus, there has been a continuing search for new and more potent antibiotics [26]. Plant derived photochemical serves as arsenal in controlling the growth of microorganisms due to their availability, fewer side effects and reduced toxicity. These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus [27]. Numerous studies have been conducted on the antimicrobial activity of different plant extracts. Many plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections [28, 29].

In the present study, extracts of *T. pallida* was evaluated for exploration of their antimicrobial activity against certain Gram negative and Gram positive bacteria which was regarded as human pathogenic microorganism. In this study, for the first time we have attempted to evaluate and compare the antimicrobial activity of different parts of *T. pallida*. Susceptibility of each part extracts was tested by serial dilution method and agar well diffusion method. Therefore, medicinal plants are finding their way into pharmaceuticals, naturalceuticals and food supplements.

Our preliminary findings showed that methanol extracts of different parts of *T. pallida* were moderately active against some human pathogens like *Escherichia coli, Staphylococcus aureus*, *Bacillus cereus*, *Shigella dysenteriae* and *Shigella sonnei, Agrobacterium species*. Our findings suggest that the flower parts of *T. pallida* showed the best antibacterial activity against all tested clinical bacterial pathogens (Table 1). Though the leaves and stem bark parts showed varying degree of antibacterial susceptibility against various strains of tested pathogens but the antibacterial susceptibility of flowers was remarkable among all parts of *T. pallida* against all susceptible tested pathogens. The study also revealed that the root part of this plant was inactive against tested pathogens. Though, the mechanism of action of these plant constituents is not yet fully known it is clear that the effectiveness of the extracts largely depends on the content and nature of their bioactive compounds that also depends on the type of solvent is used. It is reported that most of the antibiotic compounds already identified in plants are aromatic or saturated organic molecules which can easily solubilized in organic solvents [30, 31]. Our findings also indicate that the methanol extract of different parts mostly solubilizes the active compounds which was bacteriostatic and bacteriocidal in nature that shows antimicrobial activity. The MIC value of the plant extracts supports their zone of inhibition with the range of 125 to 250 µg/ml. In case of *E. coli* the MIC (125 µg/ml) value was equal to MBC (125) value of flowers part that suggests the presence of bactericidal effect. For other parts like stem and leaves the MIC value was lower than their MBC value against *E. coli* (Table 2) suggesting that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentration.

5. Conclusion

In conclusion, the antimicrobial compounds generated by these medicinal plants are active against some human pathogenic microorganisms. Among evaluated parts of *T. pallida*, flower part was possessed the most active phytochemicals and showed a broad spectrum and significant antibacterial activity against clinical bacterial pathogens. This antibacterial activity was associated with the variety of photochemical existing in these plants. Moreover the low value of MIC and MBC for the susceptible extracts against the most important clinical bacterial pathogen *E. coli* was valuable. However, it is still unclear which phytochemicals are playing vital roles for these activities. Therefore, further phytochemical and pharmacological study is necessary to isolate and characterize the bio-active compounds that can

appeared as a miracle healing agent for infectious diseases caused by globally concerning multi-resistant human pathogens.

6. References

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