



Phytochemical and Antimicrobial Properties of *Tiliacora triandra* Stem Bark

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Authors' contributions

This work was carried out in collaboration between all authors. Author Md. Mahbubur Rahman designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MS and MK managed the analyses of the study. Author Md. Mostafijur Rahman managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: *Tiliacora triandra* (*T. triandra*), a species of Menispermaceae family, has folkloric reputation for the treatment of several diseases including infectious diseases in Thailand and neighboring countries. The present study aimed at screening the stem bark of *T. triandra* for its phytochemical constituents and its antimicrobial potential against selected bacteria and fungi.

Materials and Methods: The dried stem bark of *T. triandra* was extracted with methanol and qualitative phytochemical analysis was performed. The antimicrobial activity was determined by disc diffusion assay method against some bacteria and fungi. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by serial dilution method to establish the antimicrobial potential of the extract.

Results: Qualitative phytochemical analysis revealed the presence of phenolics, flavonoids,

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terpenoids, alkaloids, saponins and cardiac glycosides. Susceptibility testing by disc diffusion assay showed significant antimicrobial activity against the tested pathogens. The highest antibacterial activity was observed against a Gram-negative bacterium *Escherichia coli* (*E. coli*) where the zones of inhibition were 15 mm and 21 mm at the potencies of 250 and 500 µg/disc, respectively. The stem bark extract also showed moderate activity against *Shigella sonnei* (14 mm), *Shigella dysenteriae* (13 mm), *Agrobacterium spp* (13 mm) bacteria and fungus, *Aspergillus niger* (14 mm) at 250 µg/disc. The results also revealed equal MIC and MBC values of 62.5µg/mL against *E. coli*.

Conclusions: Our findings revealed that the methanol extract of *T. triandra* possesses potent antimicrobial activity which may be attributed to the identified phytochemical components of the plant extract.

Keywords: Antimicrobial; phytochemical screening; *Tiliacora triandra*; minimum inhibitory concentration; minimum bactericidal concentration.

1. INTRODUCTION

Critical challenges remain in the battle against infectious diseases in spite of enormous advent of most promising healing agent, antibiotics, over the past few decades. Available evidences revealed that infectious diseases remain the third leading cause of death in USA each year and the second leading cause of death worldwide [1]. Currently, more than 100 kinds of antibiotics have been discovered and are widely used to cure various infectious diseases caused by microbes. Unfortunately, the clinical efficacy of these antibiotics is being threatened due to the emergence of multi-drug resistant (MDR) pathogens, also known as 'superbugs' which cause antimicrobial resistance [2]. Nowadays, antimicrobial resistance has been a most alarming global concern for years and is considered one of the world's most critical public health threats. Rezaei and Weinstein, (2010) reported that over 70% of pathogens found in US hospitals acquired resistance for at least one antibiotic resulting in mortality of more than 14,000 patients annually from microbial infections [3]. To overcome antimicrobial resistance challenge, it is essential to investigate new antimicrobial agents with diverse chemical structure and novel mechanisms of action for new and re-emerging infectious diseases [4]. 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 [5, 6,7]. It has been reported that natural products, either as pure compounds or as standardized plant extracts may give a new source of antimicrobial agents with possibly novel mechanisms of action [8,9]. From 1981 to 2002,

about 61% of new drugs were developed on the basis of natural products, mainly plant source and they have been very successful, especially in the areas of infectious disease and cancer [10]. Therefore, researchers have directed their efforts at screening and testing the efficacy of plants to explore their antimicrobial activity towards discovery of new leads to develop better drugs against infections caused by microbes.

Tiliacora triandra (*T. triandra*), a member of *Menispermaceae* family, is commonly known as Diels or Ya-nang especially in Thailand. It is a species of flowering plant native to Southeast Asia and widely distributed in the Northeast of Thailand and Lao PDR [11]. *T. triandra* is a climbing plant with deep green leaves and yellowish flowers. It has long history of use as edible plant in Thai cuisine, especially in bamboo shoot soup. Ya-nang is used not only as food but also as traditional medicine to treat different human diseases such as malaria, fever, cancer etc. Its leaves and root extracts contain high levels of beta-carotene and minerals and are often used as traditional medicine in the treatment of fever and cancer [11,12]. Furthermore, some bisbenzyl isoquinoline alkaloids, especially tiliacorine, tiliacoronine and nor-tiliacoronine have been identified from the root extracts of Ya-nang, which have antifungal, antioxidant, anticancer and anti-malarial activities [11,13]. Naibaho [14] reported that the essential oil isolated from fresh leaves of *T. triandra* has a strong antibacterial activity against both Gram-positive and Gram-negative bacteria. Although roots and leaves of *T. triandra* possesses biological activities against several diseases, including infectious diseases, little is known about the stem barks of *T. triandra*. Therefore, the objective of the present study was to evaluate *T. triandra* stem barks for its

antimicrobial activity against selected pathogenic microorganisms.

2. MATERIALS AND METHODS

2.1 Plant Collection

Stem barks of *T. triandra* was collected from Manda upozilla under Naogaon district, Rajshahi, Bangladesh in July, 2016 and was identified by an expert taxonomist at the Department of Botany, University of Rajshahi, where a voucher specimen (Voucher No. MR-01) was deposited. Plant materials were then washed separately with fresh water to remove dirt and other contaminants, and were shade-dried for 5 to 7 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 [15].

2.2 Extract Preparation

The extraction was performed according to the method of Rahman et al. [16]. About 150 g of powdered plant materials soaked with 500 mL of 80% methanol in an amber-coloured extraction bottle. 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 then concentrated with a rotary evaporator (Bibby Sterlin Ltd, UK) under reduced pressure at 50°C to afford 30 g extract of stem barks.

2.3 Phytochemical Screening

The extract was analyzed for the presence of phenolics, flavonoids, terpenoids, saponins, alkaloids, cardiac glycosides and protein as described by following standard methods.

2.3.1 Ferric chloride test for phenolic compounds

Each extract (about 50 mg) was dissolved in 5 mL of distilled water and few drops of 5% ferric chloride were added. Bluish black colour indicated the presence of phenolic compounds [17].

2.3.2 Alkaline reagent test for flavonoid compounds

Few drops of sodium hydroxide were added into the extracts (500 mg) to give intense yellow

colour. The disappearance of colour after addition of dilute hydrochloride acid showed the presence of flavonoid [18].

2.3.3 Salkowski's test for terpenoids

The extract (50 mg) was added with few mL of chloroform followed by concentrated sulphuric acid to form a layer. Reddish brown colour at the interface indicated the presence of terpenoids [19].

2.3.4 Froth test for saponins

Each extract (50 mg) was diluted with distilled water and made up to 20 mL. The suspension was shaken in a graduated cylinder for 15 mins. The development of 2 cm layer of foam indicated the presence of saponins [20].

2.3.5 Wagner's test for alkaloids

About 50 mg of extracts was stirred with few mL of dilute hydrochloric acid and filtered. Then, few drops of Wagner's reagent were added at the side of the test tube. The formation of reddish brown precipitate showed the presence of alkaloids [21].

2.3.6 Keller-Kiliani's test for cardiac glycosides

A small amount of extract was treated with 2 mL of glacial acetic acid containing one drop of 5% ferric chloride, followed by addition of 1 mL of concentrated sulphuric acid. A brown ring at the interface is a characteristic of cardenolide deoxy sugar. Appearance of the violet ring below the brown ring and greenish ring in acetic acid layer indicated the presence of cardiac glycosides [19].

2.3.7 Biuret test for protein

Each extract (50 mg) was diluted with distilled water and treated with Biuret reagent. The appearance of pink colour indicated the presence of protein [18].

2.4 Antimicrobial Study

2.4.1 Micro-organisms

Three Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*) and Gram -negative (*Escherichia coli*, *Shigella dysenteriae*, *Agrobacterium spp* and *Shigella sonnei*) bacteria and four fungi (*Aspergillus niger*,

Trichoderma viride, *Trichoderma harizianum* and *Micropharina phascolina*) were used in this study. The pure culture of bacteria and fungus were maintained on nutrient agar medium and potato dextrose agar medium, respectively in the microbiology lab of Pharmacy Department in Rajshahi University. Each culture was further maintained by sub-culturing regularly on the same medium and stored at 4°C before use in experiments.

2.4.2 In-vitro antibacterial activity

In-vitro antibacterial activity was carried out on nutrient agar plate using disc diffusion method [22]. The crude extract of *T. triandra* stem bark was dissolved in 1 mL of methanol. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amount of tested sample and prepared as 250 and 500 µg/disc. Discs with tested sample were placed on agar plate previously inoculated with test organisms by sterilized forceps. The inoculated agar plates were left in refrigerator for one hour for proper diffusion. The plates were then incubated at 37°C for 24 hours. Standard antibiotic discs, Kanamycin (KN) 30 µg/disc and blank discs (impregnated with solvent, methanol) were used as a positive and negative control, respectively. The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition, expressed in mm.

2.4.3 Test for antifungal activity

In-vitro antifungal activity of crude methanolic extract was carried out on Sabouraud Dextrose Agar plate by disc diffusion method against four pathogenic fungi at a concentration of 250 and 500 µg/disc as described in antibacterial screening section. Standard disk of Clotrimazole (CM) (10 µg/disc) was used as positive control.

2.4.4 Determination of minimum inhibitory concentration (MIC)

The stem bark extract that showed antimicrobial activity against respective bacterial strain was later tested to determine the MIC value. MIC referred to the lowest concentration of the extract required to inhibit the growth of the organism and performed by a serial dilution technique according to the NCCLS protocol [23]. The extract was diluted to give the final concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.91µg/mL. A volume of 10 µL of 10⁷ cell/mL of the tested microorganism was

inoculated in tubes with equal volume of nutrient broth and plant extracts. The MIC value was detected in µg/mL after 18-24 hours 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 has no visible growth (no turbidity) of respective tested organism in comparison with control was defined as MIC.

2.4.5 Determination of minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

MBC value was determined by sub-culturing the test dilution (which showed no visible turbidity) on to freshly prepared nutrient broth medium described in document M26-A [24]. 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.

The fungicidal concentrations (MFC) were also determined by serial sub-culturing the test dilution (which showed no visible turbidity) on to freshly prepared nutrient broth medium as like MBC determination. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculums.

2.5 Statistical Analysis

Microsoft Excel 2007 (Roselle, IL, USA) was used for the statistical and graphical evaluations.

3. RESULTS

3.1 Phytochemical Screening

Freshly prepared methanolic extract of *T. triandra* stem bark was subjected to preliminary phytochemical screening for various constituents. Table 1 shows the presence of phenolics, flavonoids, terpenoids, alkaloids, saponins and cardiac glycosides. Although stem bark extract exhibited differential distribution of phytoconstituents, no proteins were detected in *T. triandra*.

3.2 In-vitro Antibacterial Activity

The antibacterial potential of stem bark extract (250 µg/disc and 500 µg/disc) 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 (30 µg /disc). The stem bark extract showed prominent differential antibacterial activity against all tested microorganisms. A considerable increase in inhibitory effect was found with increased concentration of the sample. The zones of inhibition were ranging from 11 to 13 mm at 250 µg/disc, whereas at 500 µg/disc the zones of inhibition were ranging from 16 to 21 mm against Gram-positive bacteria. Moreover, Gram-negative bacteria showed 13 to 15 mm zones of inhibition at 250 µg/disc and 17 to 21mm at 500 µg/disc (Table 2). The highest zone of growth inhibition was 15 mm against *E. coli*, while the least inhibition was found to be 11 mm against *B. cereus* at the concentration of 250 µg/disc. At the concentration of 500 µg/disc, the zone of growth inhibition by the extract was 21 mm against *E. coli* where 16 mm against *S. aureus*, 21 mm against *B. cereus*, 18 mm against *Agrobacterium spp* and 18 mm against *S. dysenteriae*, were similar to that of standard antibiotic, Kanamycin (Table 2).

Table 1. Phytochemical analysis of *T. triandra* stem bark extract

Phyto-chemicals	Methanol extract
Alkaloids	+
Phenolic compounds	+
Flavonoids	+
Terpenoids	+
Saponins	+
Cardiac glycosides	+
Protein	-

+: Present; -: Absent

3.3 Antifungal Activity

Four fungi were used for antifungal activity test of the crude extract. All tested fungi were

promisingly inhibited by the stem bark extract of *T. triandra* as shown in Table 3. Among the tested fungi the *Aspergillus niger* was most susceptible with 14 mm and 22 mm zones of growth inhibition at 250 µg/disc and 500 µg/disc concentrations respectively in comparison to the standard Clotrimazole, 23 mm. Both *T. viride* and *T. harizianum* were less susceptible to the stem bark extract at the concentration of 250 µg/disc with 10 mm zones of growth inhibition. At 500 µg/disc concentration, the zone of growth inhibition against *M. phascolina* (21 mm) was similar to the standard antibiotic Clotrimazole (21 mm) shown in Table 3.

3.4 Determination of MIC, MBC and MFC Values

The MIC, MBC and MFC values obtained for the methanol extract against the tested pathogenic microorganisms was remarkable. The results shown in Table 4 shows that methanol extract of *T. triandra* stem bark possesses potent antimicrobial activity. The least MIC was produced by the stem bark extract against *E. coli* (62.5 µg/mL), while the highest value was recorded at concentrations greater than 125 µg/mL against *B. cereus*. The MIC and MBC values (Table 4) for the stem bark extract of *T. triandra* were same (62.5 µg/mL) against *E. coli*. However the MBC value was higher than the MIC value against other tested organisms.

The extract of *T. triandra* also showed potent antifungal activity as compared to their antibacterial efficacy. The antifungal MIC values range between 62.5 to 125 µg/mL. Among the tested fungi, *M. phascolina* was highly susceptible with lowest MIC value (62.5 µg/mL) compared to other tested fungi but MFC values against all tested fungi were higher than their MIC values (Table 4).

Table 2. Antibacterial activity (zone of inhibition, mm) of methanol extract of *T. triandra* stem bark

Types of organisms	Name of organisms	Zone of inhibition at 250 µg/disc	Zone of inhibition at 500 µg/disc	Kanamycin (30 µg /disc)
Gram positive Bacteria	<i>Bacillus cereus</i>	11	21	21
	<i>Staphylococcus aureus</i>	12	16	16
Gram negative Bacteria	<i>Escherichia coli</i>	15	21	22
	<i>Shigella dysenteriae</i>	13	18	18
	<i>Shigella sonnei</i>	14	17	18
	<i>Agrobacterium spp</i>	13	18	18

Table 3. Antifungal activity (zone of inhibition, mm) of methanol extract of *T. triandra* stem bark

Types of organism	Name of organisms	Zone of inhibition at 250 µg/disc	Zone of inhibition at 250 µg/disc	Clotrimazole 10 µg/disc
Fungus	<i>Aspergillus niger</i>	14	22	23
	<i>Trichoderma viride</i>	10	18	19
	<i>Trichoderma harzianum</i>	10	15	20
	<i>Microphamina phascolina</i>	13	21	21

Table 4. MIC (µg / ml), MBC and MFC performance of methanol extract of *T. triandra* stem bark against pathogenic organisms

Types of organisms	Names of organisms	MIC (µg/ml)	MBC / MFC (µg/ml)
Gram positive Bacteria	<i>Bacillus cereus</i>	>125	>125
	<i>Staphylococcus aureus</i>	125	>125
Gram negative Bacteria	<i>Escherichia coli</i>	62.5	62.5
	<i>Shigella dysenteriae</i>	125	>125
	<i>Shigella sonnei</i>	125	>125
	<i>Agrobacterium spp</i>	125	>125
Fungus	<i>Aspergillus niger</i>	125	>125
	<i>Trichoderma viride</i>	125	>125
	<i>Trichoderma harzianum</i>	125	>125
	<i>Microphamina phascolina</i>	62.5	>62.5

4. DISCUSSION

In recent years, an explosive spread of multi-drug resistant bacterial pathogens (superbugs) has become a serious concern worldwide in terms of public health and economic effects. Therefore, antimicrobial screening for the discovery of novel antimicrobial agents from natural sources has gained immense attention, and the antimicrobial activity attributed to some plants in treating diseases has been beyond belief. Recently, scientists have focused their researches and efforts on identifying a drug or phytoconstituent having antimicrobial property which might be considered suitable antimicrobial agent to replace synthetic ones. Numerous studies have been conducted on the antimicrobial activity of different plant extracts. Plant-derived phytochemical serve as arsenal in controlling the growth of microorganisms due to their availability, fewer side effects and reduced toxicity. These compounds have significant therapeutic applications against human pathogens including bacteria, fungi and/ virus [25]. Many plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections [26,27].

In the present study, methanol extract of *T. triandra* stem bark was evaluated for its

phytoconstituents and antimicrobial activity against selected Gram-positive and Gram-negative bacteria and fungi which were regarded as human pathogens. Susceptibility of each of the test microorganisms to *T. triandra* stem bark extract was evaluated using serial microdilution method (MIC) and disc diffusion method.

Our preliminary phytochemical screening of the stem bark extract of *T. triandra* showed the presence of phenolic, flavonoids, terpenoids, alkaloids, saponins and cardiac glycosides (Table 1). These biologically active constituents are known to act by different mechanisms with some of which have been previously associated with antibacterial activity [28,29]. Plants possessing alkaloids have analgesic, antispasmodic and bactericidal effects. Plant phenolic compounds impart colour, flavour and are associated with health benefits such as reduced risk of heart and cardiovascular diseases due to their antioxidant properties. Flavonoids are hydroxylated phenolic substances synthesized by plants and have been found *in-vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins as well as bacterial cell walls [30,31]. Correspondingly, terpenoids such as triterpenes, sesquiterpenes and diterpenes have been used as antibiotics,

insecticides, anthelmintic and antiseptic in pharmaceutical industry. Plant saponins possess antimicrobial property due to their ability to cause leakage of proteins and certain enzymes from the cell [32,33].

Antimicrobial evaluation of methanol extract of *T. triandra* stem bark revealed significant antimicrobial activity against some human pathogens like *Escherichia coli*, *Shigella sonnei*, *Agrobacterium spp*, *Staphylococcus aureus*, *Bacillus cereus*, *Shigella dysenteriae* as well as few fungi. Previous studies reported antimicrobial properties of *T. triandra* extracts of leaves, roots and few specific phytoconstituents obtained from *T. triandra* [14,34,35]. Our results support the previous reports of the antimicrobial potential of this species. It can be attributed that, the stem bark extracts of *T. triandra* possesses antimicrobial activity largely depends on the content and nature of their phytochemical 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 [36,37]. In the present study, the MIC values ranging from 62.5 to 125 µg/mL (Tables 2, 3 and 4) of the plant extract were lower than the MBC values suggesting that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentration against respective tested pathogens. However, the MIC value of the extract against *E. coli* was equal to MBC value (62.5 µg/mL) suggests that the *T. triandra* has bactericidal effect.

5. CONCLUSION

The results of preliminary phytochemical screening suggest that methanol extract of *T. triandra* stem bark is a good source of beneficial phytochemicals. These phytochemicals exhibits their broad spectrum and strong antibacterial as well as antifungal activity against selected test microorganisms. Therefore, further phytochemical and pharmacological studies are necessary to isolate and characterize the bio-active compounds responsible for the observed activity in this study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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