Original Research Article

Antibacterial Potentiality and Brine Shrimp Lethality Bioassay of the Leaves Extract of *Trema orientalis*

Abstract

Aim: The aim of this study was to evaluate the antibacterial and cytotoxic activities of methanol extract of leaves of *Trema orientalis*.

Materials and Methods: Antibacterial activity of leaves of *Trema orientalis* was tested against two Gram positive and seven Gram negative bacteria by disc diffusion assay. The liquid micro dilution assay was used for the determination of the minimum inhibitory concentration (MIC). The cytotoxic activity of methanol extract of leaves of *Trema orientalis* was analyzed by brine shrimp lethality bioassay.

Results: The methanol extract exhibited potent antibacterial activity with the zone of inhibition ranging from 9 to 14 mm against both the tested Gram positive and all tested Gram negative bacteria except *Pseudomonus denitrificans* and *Xanthomonas campestris.* Comparatively, higher antibacterial activity was found against Gram negative bacteria in case of *Shigella dysenteriae* and *Salmonella typhi* showed 14 mm and 13 mm zones of inhibition respectively. *Salmonella typhi* showed resistance against reference antibiotics (Tetracycline, Erythromycin, Gentamicin and Ciprofloxacin) but methanol extract of leaves exhibited potent antibacterial activity against *Salmonella typhi.* The MIC values for tested Gram positive bacteria was 10 mg/mL while for Gram negative bacteria were ranged from 1.25 to 20 mg/mL. Methanol extract of *Trema orientalis* leaves showed very low cytotoxicity (LC50, 170.215 µg/mL) in comparison with the standard vincristine sulphate having LC_{50} value 2.477 µg/mL.

Conclusion: The results suggest that the methanol extract of *Trema orientalis* leaves has potent antibacterial activity with minimum cytotoxicity and could lead to the development of novel broad spectrum antibacterial agent.

Keywords: *Trema orientalis,* Antibacterial activity, Cytotoxicity, Disc diffusion, Minimum inhibitory concentration and Brine shrimp lethality bioassay.

1. Introduction

Pathogenic microorganisms, such as bacteria, viruses, parasites and fungi cause infectious diseases. The disease can transmit directly or indirectly from one person to another. One half of all death in tropical countries is caused due to infectious diseases. The infectious diseases are considered as major threat to human health [1] and dangerous for child and young adults and responsible for the second leading cause of death worldwide [2]. Bacterial infections have again become a threat after many decades and the first patient treated with antibiotics. The Gram positive and Gram negative bacteria like different species of *Bacillus, Escherichia, Proteus, Klebsiella*, *Xanthomonas*, *Pseudomonas* are main source to cause severe infection in humans. Because these organisms are capable to survive in adverse condition due to their multiple environmental habitats [3]. The synthetic antibiotics are costly and are out of range from the patient belonging to developing countries. With the passage of time, microorganisms develop resistance against antibiotics and the antibiotic resistance climax has been attributed to the indiscriminate and inappropriate use of these medications as well as a lack of new drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements [4,5,6,7]. In addition, almost all of the antibiotics have side effects on the host including allergic reactions, immune-suppression and hypersensitivity [8, 9]. New and re-emerging infectious diseases are rising very rapidly. Due to these problems, attention is now being given to biologically active compounds isolated from plant species commonly used as herbal medicine as they offer a new source of antibacterial, antiviral and antifungal activities and are widely perceived as natural and safe, that is, not toxic [9, 10]. Moreover, plant-based medicines contain diverse chemical structure and novel mechanism of action that work in a way of orchestral ensembles which are able to

target many elements of the complex cell signaling pathways [11]. Plant-based antibiotics such as Quinine (Cinchona) and berberine (Berberis) are highly effective against *Staphylococcus aureus* and *Escherichia coli* [9]. Therefore, there is an urgent need to search new and more potent anti-bacterial and bioactive agents that can fight against those pathogen.

T*rema orientalis* has common name such as pigeon wood, charcoal tree, Indian charcoal tree, and gunpowder tree. It is the fast growing and evergreen tree with soft foliage belongs to the family Ulmaceae and distributed all over the world in countries such as Bangladesh, Angola, Australia, Brunei, Cambodia, Ghana, Senegal, Sierra Leone, Niger, Cote d'Ivoire, Cameroon, Central African Republic, Chad, China, Democratic Republic of Congo, Ethiopia, India, Indonesia, Japan, Kenya, Laos, Madagascar, Malaysia, Mali, Myanmar, Nepal, Nigeria, Philippines, Saudi Arabia, South Africa, Sudan, Zimbabwe, Tanzania, Uganda, Vietnam and Zambia. It can grow on a wide range of soils from heavy clay to light sand [12]. *Trema orientalis* is a shrub or small to medium size tree. It has wide variety of sizes depending on the location and climatic conditions and grows up to 18 m high in forest regions and up to 1.5 m tall in the savannah. The slender branchlets have white velvety hairs. The extensive root system of *Trema orientalis* enables it to survive long periods of drought. The base is frequently unequal and the leaves are alternate, simple and stipulate although the stipules drop early. margins are finely serrulated whereas the young leaves are rough and hairy, occasionally becoming smooth when old. The inconspicuous flowers are small and greenish, carried in short dense bunches and appear irregularly from late February to April. The round fruits are small and dark purple or green drupes that become black upon ripen and carried on very short stalks [13]. The young leaves are eaten as spinach and the roots and stem bark are used as traditional medicine by the Zulus in South Africa [14]. In combination with lemon juice, the leaves maceration are used for the treatment of bronchitis, cough, pneumonia and

pleurisy. The decoction of leaves is also used as an anti-helminthic medicine for hookworms and roundworms in East Africa, West Africa, Madagascar and some parts of Central Africa. The infusion is prepared from fruits and flowers of *Trema orientalis* for administration to children as a therapy for pneumonia, pleurisy and bronchitis [15]. The decoctions of stem bark and are used as vermifuge and to treat malaria and dysenteries, manage pain in tired muscles and aching bones as well as venereal disease [16-20]. The root is used in folk medicine for treatment of blood stasis, trauma, hematuria and bleeding of intestines and stomach [21]. Numerous research report claimed that the different pharmacological effects of *T. orientalis* in various test models. The aerial parts, flowers, bark, and seeds of *T. orientalis* exhibit various pharmacological activities including hypoglycemic, analgesic, anti-inflammatory, laxative, anti-plasmodial, diuretic anti-convulsant anti-helmintic, anti-sickling, anti-oxidant and anti-bacterial activities. These pharmacological activities may be mainly due to the fact that it contains important biologically active compounds such as scopoletin, 4-hydroxybenzoic acid, Epicatechin, lupeol, methylswertianin, catechin, hexacosanoic acid and 3,4-dihydroxybenzoic acid [22-24]. Various secondary metabolites like tannins, saponins, flavonoids, triterpenoid (simiarenol, simiarenone, trematol) phytosterols, and several constituents of xanthones has been isolated from and stem bark of this plant [12] and these metabolites may be responsible for cytotoxicity and antibacterial activities. Although there are many literatures reporting the ethno-medicinal values of *T. orientalis* there is little scientific proof for further using this plant commercially or in a more effective form. Therefore, an attempt was made to evaluate the antibacterial and cytotoxic activities of the crude methanol extract of *T. orientalis* leaves to support the pharmacological effects and phytochemical investigation of the plant.

2. Materials and Methods

2.1. Plant Material

The leaves of *Trema orientalis* were collected during the month of January 2015 from Jessore University of Science & Technology, Jessore-7408, Bangladesh. This plant was then botanically identified by Bushra Khan, Principal Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka 1216, Bangladesh. A voucher (DACB 31285) has been deposited in Bangladesh National Herbarium, Mirpur, Dhaka 1216, Bangladesh. The collected plant leaves were washed with running tap water and dried in shade at room temperature. The air dried leaves were pulverized into fine powder by commercial blender (Philips, South Korea) and stored in sealed container.

2.2. Experimental Methods

2.2.1. Preparation of Extract

One hundred gram of powder was taken in a 500 ml conical flask added with 350 mL of methanol. The flask was kept for 7 days with continuous shaking at shaking incubator at room temperature. The plant extract was filtered into beaker through Whatman no.1 filter paper to exclude the insoluble powder. The extract was then concentrated by using a rotary evaporator and kept at room temperature to evaporate remaining solvent. After complete evaporation of solvent, only plant's crude extracts were obtained. The amount of crude extracts was 1.0 g which was stored in refrigerator at 4°C in sterile container for further use.

2.2.2. Tested Bacterial Preparation

Pure culture of Gram positive bacteria (*Bacillus subtilis* IFO 3026, *Sarcina lutea* IFO 3232) and Gram negative bacteria (*Escherichia coli* IFO 3007,*Proteus vulgaris* MTTC 321, *Klebsiella pneumonia* ATTC 10031, *Xanthomonas campestris* IAM 1671, *Pseudomonas denitrificans* KACC 32026) were used in this study and obtained from the Microbiology

Laboratory of Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh. Another two Gram negative bacteria, *Salmonella typhi* and *Shigella dysenteriae* were kindly provided by the Microbiology laboratory of Department of Microbiology, Jessore University of Science &Technology. Bacteria were cultured in Nutrient agar media and Nutrient broth media. For antibacterial assay, minimum inhibitory concentration (MIC) determination and the further stock culture preparation, 100 µL of frozen stock culture was inoculated into 125 mL conical flask containing 25 mL of Nutrient broth media and incubated at 37°C with continuous shaking at 250 rpm for culturing the bacteria until mid-log phase of absorbance at 600 nm reached at 0.4 by using UV spectrophotometer (Oasis scientific Inc., USA) for bacterial broth culture.

2.2.3. Disc Preparation

The Whatman No. 1 filter paper discs (6 mm diameter) were transferred to a small vial and autoclaved at 15 lb/inch^2 pressure for 15 minutes at 121°C. The discs were completely dried in drying oven at 60°C. Four hundred mg of crude methanol extract of *Trema orientalis* was dissolved into 10 mL of methanol and each disc was impregnated with 10 µL of 40 mg/mL (400µg/disc) of *Trema orientalis* leaves extract. The discs were completely air dried in the laminar flow cabinet and used for antibacterial assay. Blank discs (negative controls) impregnated with 10 µL of methanol.

2.2.4. Antibacterial Activity Assay

Antibacterial activity of crude extract was tested by the disc diffusion method [25]. The prepared discs were placed on nutrient-agar-medium plate spreaded with100 µL of tested bacterial broth culture and the plates were incubated at 37°C for 24 h. Standard reference antibiotics Tetracycline (30 µg/disc), Erythromycin (15 µg/disc), Gentamicin (10 µg/disc) and Ciprofloxacin (5 µg/disc) were used as positive control to ensure the activity of standard

antibiotic against the test organisms. The blank discs were used as negative control. After incubation, the culture plates were examined and the inhibition zones formed around each disk were measured in millimeter scale as previously described [26]. Each assay in this experiment was replicated three times.

2.2.5. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) of methanol extract of of *Trema orientalis* was determined by a two-fold serial dilution method as previously described [27]. The methanol crude extract of *Trema orientalis* leaves was dissolved in Nutrient broth medium in an eppendorf tube to achieve a concentration of 40 mg/mL. The solution of eppendorf tube was serially diluted to obtain 20, 10, 5, 2.5 and 1.25 mg/mL of concentrations. The 0.5 mL of bacterial broth culture of each tested bacteria was transferred to each eppendorf tube. Thus, the total amount of solution in each eppendorf tube was 1 mL. The control tubes contain 0.5 mL bacterial broth cultures with 0.5 mL nutrient broth media. The solution of all eppendorf tubes were mixed properly by vortexing and incubated at 37°C for 24 h with continuous shaking at 250 rpm. After incubating 24 h, 100 µL of solution from each eppendorf tube were spreaded over the nutrient-agar-media plate. The plates were incubated at 37°C for 16 h for bacterial growth and the number of colony was counted for MIC determination.

2.2.6. Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay is the most convenient system for preliminary assessment of cytotoxicity of plant extracts. The brine shrimp lethality bioassay of the methanol extract of of *Trema orientalis* was evaluated as previously described procedure against *Artemia salina* as a test organism to monitor the cytotoxicity of a compound [28]. The eggs of Brine shrimp (*Artemia salina*) were collected from an aquarium shop (Dhaka, Bangladesh) and incubated

for 28°C with constant oxygen supply and hatched for two days to provide a large number of larvae called nauplii. The different concentrations of crude extract were prepared by dissolving them in DMSO (not more than 50 µL in 5 mL solution) plus seawater (3.8% NaCl in water) to attain concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.562 µg/mL. The standard vincristine sulphate was used as a positive control. The varying concentration of solution of vincristine sulphate was prepared by serial dilution into DMSO to attain a concentration of 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.562 µg/mL. A vial containing 50 µL of DMSO diluted to 5 mL simulated seawater used as a control. Ten mature shrimp were placed into each of the experimental vials. After 24 h, the vials were inspected using a magnifying glass, and the number of surviving nauplii in each vial was counted. From this data, the percentage (%) of mortality of the brine shrimp naupili was calculated for each concentration using the following formula: % Mortality = N_t/N_0 x 100 (Where N_t = Number of dead nauplii after a 24 h incubation; N_0 = Number of total nauplii transferred i.e., 10). The LC50 (median lethal concentration) was determined from the log concentration versus % mortality.

2.2.7. Statistical Analysis

Each experiment was replicated three times and data were reported as mean ±SEM. LC50 values were determined by correlation/regression analysis through Microsoft office excels 2007 and Statistical Package for the Social Sciences (SPSS).

3. Results

3.1. Antibacterial Potentialities of *Trema orientalis* Extract

The antibacterial activity of methanol extract of of *Trema orientalis* against the tested bacteria were examined by the occurrence of clear zone of inhibition. The leaves extract at a

concentration of 400 µg/disc showed significant antibacterial effects against two Gram positive bacteria (*Bacillus subtilis*, *Sarcina lutea*) and five Gram negative bacteria (*Escherichia coli, Salmonella typhi, Shigella dysenteriae, Klebsiella pneumoniae, and Proteus vulgaris)* with the zone of inhibition ranging from 9 to 14 mm (Figure 1). The highest zone of inhibition was found 14 mm and 13 mm against *Shigella dysenteriae and Salmonella typhi*. The inhibition zone was observed 10 mm against *Klebsiella pneumoniae, Proteus vulgaris and Sarcina lutea whereas* zone of inhibition was 9 mm against *Bacillus subtilis and Escherichia coli.* However, no antibacterial activity was observed against two Gram negative bacteria, *Xanthomonas campestris* and *Pseudomonas denitrificans* at the used concentration of 400µg/disc of plant extract. Standard reference antibiotics Tetracycline, Erythromycin, Gentamicin and Ciprofloxacin were used as positive control showed higher antibacterial activities than the plant extract against all the tested bacteria except *Salmonella typhi.* Though *Salmonella typhi* showed resistance against reference antibiotics as positive control, methanol extract of leaves of *Trema orientalis* exhibited strong zone of inhibition (13 mm) against *Salmonella typhi* (Figure 1) suggest that it could be a potential therapeutic drug candidate against *Salmonella typhi.* No zone was formed by negative control.

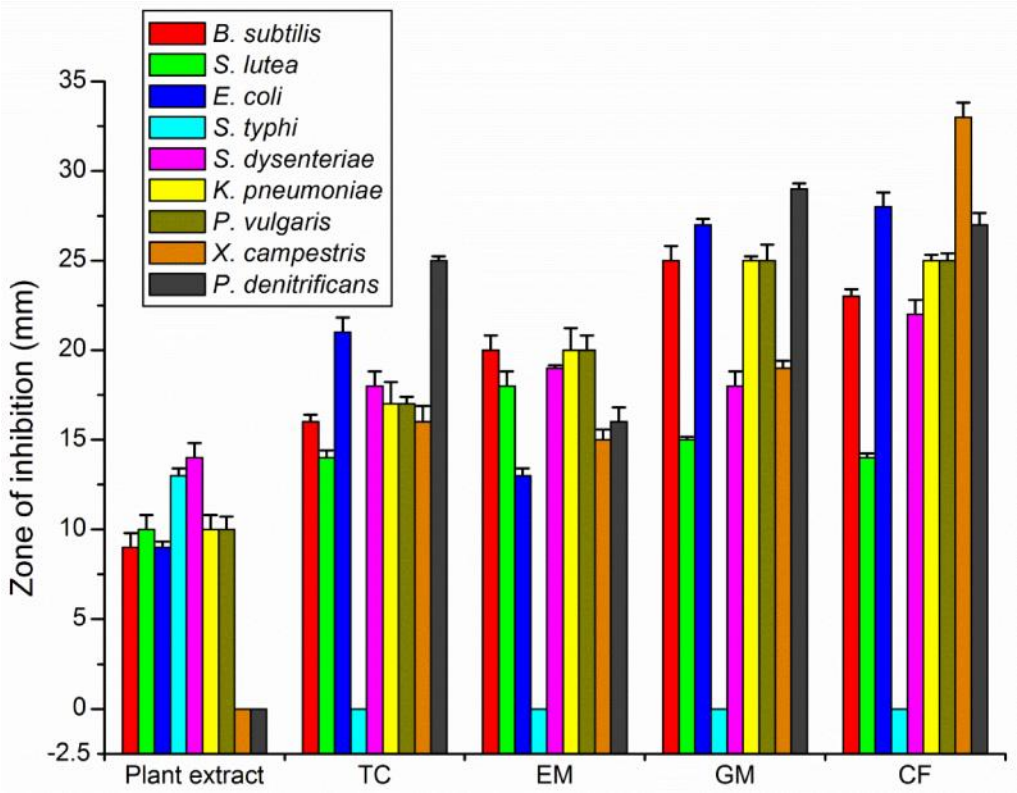

Figure 1. Effect of methanol extract of leaves of *Trema orientalis* on two Gram positive and seven Gram negative bacteria. Values are represented as mean ± SEM (n=3). TC, Tetracycline; EM, Erythromycin; GM, Gentamycin; CF, Ciprofloxacin.

3.2. Minimum Inhibitory Concentration

The lowest concentration of methanol extract of which prevent visible growth of bacterium is the minimum inhibitory concentration. The MIC values of crude extract of *Trema orientalis* were found ranging from 1.25 to 20 mg/mL (Table 1). The best MIC was 1.25 mg/mL against *Escherichia coli*, *Salmonella typhi* and *Shigella dysenteriae* as this concentration completely inhibited the growth of these bacteria. The least efficacy was shown against *Proteus vulgaris* and *Klebsiella peumoniae* which was inhibited at 20 mg/mL concentration. The moderate MIC value was shown against Gram positive bacteria (*Bacillus subtilis* and *Sarcina lutea*) which were inhibited at 10 mg/mL concentration.

238 **Table 1:** Minimum inhibitory concentration of methanol extract of leaves of *Trema*
239 *orientalis*.
240

Tested bacteria	Minimum Inhibitory Concentration (mg/mL)						
	20	10	5	2.5	1.25	0.625	0.312
	Number of bacterial colonies survived at above concentration						
Bacillus subtilis	0	0	7± .81	17± 1.41	54±1.63	96±2.16	121±.81
Sarcina lutea	0	0	12±1.41	43±2.16	66±.81	107±2.94	144±1.63
Escherichia coli	0	0	0	0	0	47±.81	133±2.16
Salmonella typhi	0	0	0	0	0	77±1.41	124±4.96
Shigella dysenteriae	0	0	0	0	0	55±.81	112±1.41
Klebsiella peumoniae	0	5±2.82	16±.81	52±1.41	79±.81	123±1.41	223±.81
Proteus vulgaris	0	23±2.16	54±.81	77±1.41	91±.81	155±1.41	175±.81

241
242 Values are represented as mean ± SEM (n=3).
243

244 **3.3. Cytotoxic Activity of Methanol Extract of *Trema orientalis***

245 The percent mortality of brine shrimp naupli at different concentrations of plant extracts and
246 vincristine sulfate as positive control are shown in Figure 2. It is clear that percent mortality
247 of brine shrimp naupli is proportional to the concentration of extracts. The mortality rate was
248 increased with the extract concentration increased. As shown in Table 2, methanol extract of
249 *Trema orientalis* demonstrated a LC50 value of 170.215 µg/mL whereas vincristine
250 sulphate showed the LC50 value of 2.477 µg/mL. This indicates that the plant leaves extract
251 has much more higher LC50 value compared to that of vincristine sulphate. The crude
252 methanol extracts resulted in LC50 values greater than 100 µg/mL were considered non-toxic
253 in the brine shrimp lethality assay [29], support the notion that the methanol extract of leaves
254 of *Trema orientalis* is non-toxic for host and had the potential for further investigation. There
255 was no mortality in the negative control groups indicating the test as a valid one and the
256 results obtained are only due to the activity of the tested agents.

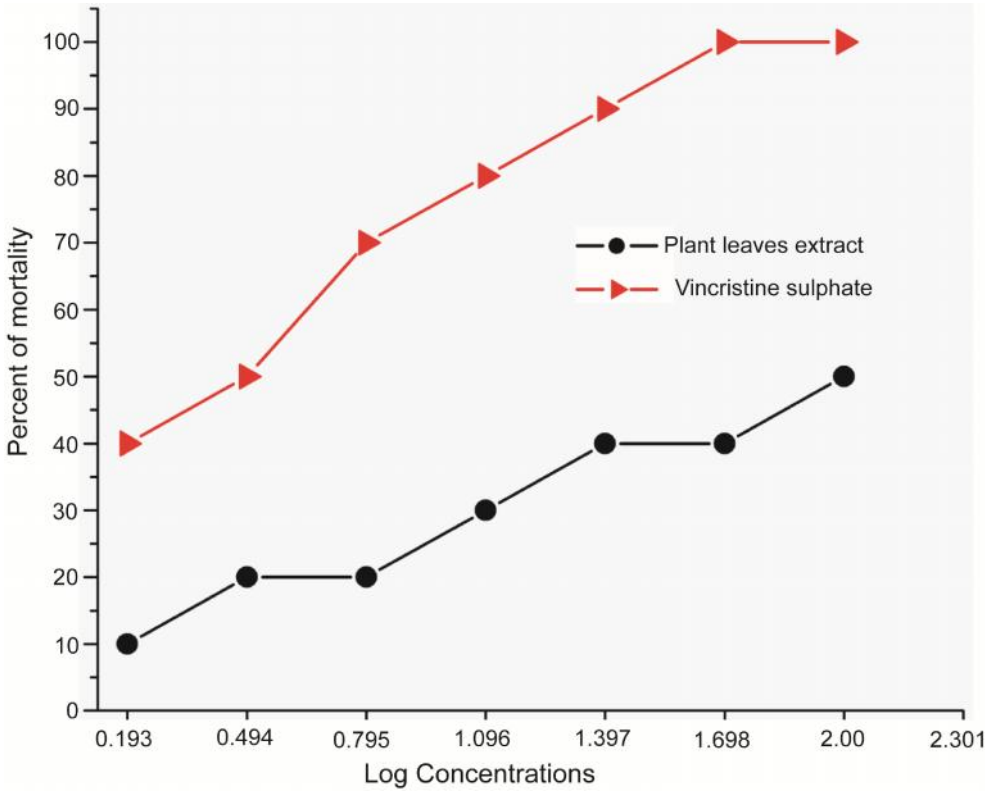

Figure 2. Brine shrimp lethality for methanol extracts of leaves of *Trema orientalis* and vincristine sulphate from linear correlation between log concentrations versus % mortality.

Table 2. The cytotoxicity of methanol extract of *Trema orientalis* leaves and vincristine sulphate on brine shrimp nauplii.

Sample	LC_{50} (µg/mL)	Regression equation	R^2
Plant extract	170.215	y=29.851x + (-16.592)	0.785
Vincristine sulphate	2.477	y=33.004x+36.976	0.838

4. Discussion

Infectious diseases are among the second leading causes of death worldwide. Recently, the emergence of antibiotic-resistant infection are rising very rapidly, are major threat to human health as well as economic burden on country's healthcare system, patients and families. The effectiveness of many conventional antibiotics is being endangered by the rapid emergence of

microbial resistance to current therapeutic agents because of their overuse, misuse, and a lack of new drug development by the pharmaceutical industry [30-34]. The peoples who live in low-income and developing countries are deprived of the advantages of modern medicine because of its high cost; hence, poor people are more vulnerable to infectious diseases. They are also in high risk of health hazards due to food hazards include microbial hazards, pesticide residues, misuse of additives, chemical contaminants, including biological toxins and adulteration [35]. Moreover, co-infection with multiple diseases is an obstacle to infection prevention and treatment. Scientists are searching for more effective medications to meet such challenges of infectious diseases [4, 30]. For all these reasons, there is an urgent need to identify new, safe and cost-effective antimicrobial agents which would help to alleviate the problems of infectious diseases. Plant-derived natural secondary metabolites represent a potential source of antimicrobial agents because they are natural; have fewer side effects and affordable especially to low-income peoples of rural society [36]. Acceptance of medicines from natural plant product as an alternative form of healthcare system is increasing because they are serving as promising sources of novel antibiotic prototype. Medicinal plant contain a large variety of chemical substances which have different mode of action than conventional drug and could be of clinical importance to improve health care [37, 38]. In developing countries, most of the people live in rural areas almost exclusively use traditional medicines to treat many diseases. Therefore, we investigated the *Trema orientalis* leaves for its antibacterial and cytotoxic activities. The present study showed that the methanol extract of *Trema orientalis* leaves at a concentration of 400 µg/disc has potent antibacterial activity against some human pathogenic bacteria. The ability of plant extract to kill or inhibit pathogenic bacterial growth indicates the presence of some active compounds which have antibacterial activity.

Since the methanol extract of *Trema orientalis* is found to exhibit anti-bacterial activity, the magnitude of toxicity of *Trema orientalis* extract is safe or acceptable at the therapeutic doses must be considered. Plant samples with a lower LC50 value are considered more toxic in nature. Extracts are considered non-toxic if the LC50 is greater than 100 µg/mL in the brine shrimp lethality assay [6]. Therefore cytotoxic assay was conducted in this study to determine the toxicity profile of methanol extract of of *Trema orientalis* through the brine shrimp lethality bioassay. Results of brine shrimp cytotoxicity were shown in Table 2, where the LC_{50} value is 170.215 µg/mL. This indicates that methanol extract of *Trema orientalis* is not toxic for host and can be a good source of potential antibacterial agents. It has been reported that crude methanol and a aqueous root extracts of *T. orientalis* has anti-bacterial activity against Gram-positive and Gram□negative bacteria. However, to the best of our knowledge, no detailed scientific proof for anti-bacterial and cytotoxic activities of leaves of *Trema orientalis* available yet for further using this plant for the development of potential new drugs or use in a more effective form. It has been well documented that the antimicrobial compounds are abundantly present in medicinal plants [39, 40]. The antimicrobial activities we showed in this study may be due to the combined effects of tannins, saponins, flavonoids, triterpenoid, phytosterols and several constituents of xanthones present in this plant. Methanol is a polar solvent is able to extract more of the extractives than other non-polar solvents (petroleum ether, chloroform) and aqueous. The result of this work however agrees with the findings of many researches, which showed that the methanol extract of plant product was active against *Bacillus subtilis, Sarcina lutea, Escherichia coli, Salmonella typhi, Shigella dysenteriae* and *Proteus vulgaris* [41,42]. The results of the minimum inhibitory concentration showed that the methanol extract of *Trema orientalis* leaves have potent bactericidal properties against the tested organisms except *Xanthomonus campestris* and *Pseudomonus denitrificans*. In comparison with commercial antibiotics, the significant

antibacterial activity was observed from the extract. It is noticeable that *Salmonella typhi* showed antibiotic resistance against all the tested commercial antibiotics but *Trema orientalis* leaves extract showed potent inhibitory effect against *Salmonella typhi* with MIC values of 1.25 mg/mL indicate the necessity of natural plant products to combat against growing resistance of bacteria. The antibacterial activity is believed to be due to the presence of secondary metabolites. The findings of this study indicates that the extract could be used against infections caused by the tested bacteria and showed a good correlation between the reported use of *Trema orientalis* in traditional medicine against infectious diseases.

5. Conclusion

The findings of present study has revealed that the methanol extract of *Trema orientalis* leaves has great antibacterial potentiality due to the presence of the compounds with high antibacterial properties that can be a source of natural antibacterial agents in developing new drugs as an alternative to synthetic bactericides. The cytotoxic activity exhibited by the plant leaves was within the permissible limit. Isolation and characterization of the active compounds could lead to a better understanding of the antibacterial mechanism for potential drug candidates for the infectious diseases in future.

References

1. Assob JCN, Kamga HLF, Nsagha DS, Njunda AL, Nde PF, Asongalem EA, et al. Antimicrobial and toxicological activities of five medicinal plant species from Cameroon traditional medicine. BMC Complement Altern Med. 2011; 11: 70. doi:10.1186/1472-6882-11-70.
2. Olajuyigbe OO, Afolayan AJ. Synergistic Interactions of methanolic extract of Acacia mearnsii de Wild. with antibiotics against bacteria of clinical relevance. Int J Mol Sci. 2012; 13(7): 8915-8932.

345 3. Ahameethunisa Ar, Hoper W. Antibacterial activity of Artemisia nilagirica
346 extract against clinical and phytopathogenic bacteria. BMC Complement
347 Altern Med. 2010; 10:6. doi:10.1186/1472-6882-10-6.
348 4. Spellberg B, Gilbert DN. The future of antibiotics and resistance: a tribute to a
349 career of leadership by John Bartlett. Clin Infect Dis. 2014; 59(S2):S71–S75.
350 5. Gould IM, Bal AM. New antibiotic agents in the pipeline and how they can
351 overcome microbial resistance. Virulence 2013;4(2):185-191.
352 6. Wright GD. Something new: revisiting natural products in antibiotic drug
353 discovery. Can J. Microbiol. 2014;60(3):147-154.
354 7. Sengupta S, Chattopadhyay MK, Grossart HP. The multifaceted roles of
355 antibiotics and antibiotic resistance in nature. Front Microbiol 2013;4:47. doi:
356 10.3389/fmicb.2013.00047.
357 8. Londonkar RL, Madire Kattegouga U, Shivsharanappa K, Hanchinalmath JV:
358 Phytochemical screening and in vitro antimicrobial activity of *Typha*
359 *angustifolia* Linn leaves extract against pathogenic gram negative
360 microorganisms. J Pharm Res 2013, 6(2):280–283.
361 9. Bibi Y, Nisa S, Chaudhary FM, Zia M. Antibacterial activity of some
362 selected medicinal plants of Pakistan. BMC Complement Altern Med 2011,
363 11:52. doi:10.1186/1472-6882-11-52.
364 10. Maiyo ZC, Ngure RM, Matasyoh JC, Chepkorir R: Phytochemical
365 constituents and antimicrobial activity of extracts of three *Amaranthus* plant
366 species. Afr J Biotechnol 2010; 9(21):3178-3182.
367 11. Lalrinzuali K, Vabeiryureilai M, Jagetia GC. Investigation of the anti-
368 inflammatory and analgesic activities of ethanol extract of stem bark of

Sonapatha Oroxylum indicum in vivo. Int J Inflam. 2016. doi.org/10.1155/2016/8247014.

12. Adinortey MB, Galyuon IK, Asamoah NO. *Trema orientalis* Linn. Blume: A potential for prospecting for drugs for various uses. Pharmacogn Rev. 2013; 7(13): 67-72.
13. Natural resources and the human environment for food and agriculture in Africa, FAO Environment and Energy Paper 1986;6:88.
14. Coates PK. Trees of Southern Africa. 1st ed. Cape Town, Johannesburg: C. Struik Publishers; 1977.
15. Watt JM, Breyer-Brandwijk MG. The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd ed. London: Livingstone; 1962; 982-7.
16. Akendengue J. Entheogenic drugs, their plant sources and history. J Ethnopharmacol 1992;37:165.
17. Githens TS. Drug plants of Africa. African Handbooks. University of Pennsylvania Press, Lancaster Press, Lancaster, United States 1948; 8:125.
18. Ayensu ES. Medicinal plants of West Africa. Nordic J Bot 1978; 4:1-3.
19. Rasoanaivo P, Petitjean A, Ratsimamanga-Urverg S and Rakoto-Ratsimamanga A. Medicinal plants used to treat malaria in Madagascar. J Ethnopharmacol 1992;37:117-27.
20. Bhat RB, Ttejere EO, Oladipo VI. Ethnobotanical studies from Central Nigeria. Econ Bot 1990;44:382-90.
21. Hines DA, Eckman K. Indigenous multipurpose trees for Tanzania: Uses and economic benefits to the people, Cultural Survival Canada and Development Services Foundation of Tanzania; Ottawa Ontario Canada 1993; 26-27.

22. Dimo T, Ngueguim FT, Kamtchouing P, Dongo E, Tan PV. Glucose lowering efficacy of the aqueous stem bark extract of *Trema orientalis* (Linn.) Blume in normal and streptozotocin diabetic rats. Pharmazie 2006; 61:233-6.
23. N'guessan K, TiebreM-S, Ake-Assi E and Zirihi GN. Ethnobotanical study of plants used to treat arterial hypertension, in traditional medicine, by Abbey and Krobou populations of agboville (Cote-d'Ivoire). Eur J Sci Res 2009;35:85-8.
24. Panchal HS, Master SM, Shah UD, Saluja AK, Dholwani KK. Anti-convulsion activity of of *Trema orientalis.* Int J Pharm Res 2010; 2:53-5.
25. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45(4):493-6.
26. Rahman MM, Rahman MM, Akhter S, Jamal MAHM, Pandeya DR, Haque MA, et al. Control of coliform bacteria detected from diarrhea associated patients by extracts of *Moringa oleifera.* Nepal Med Coll J 2010; 12(1): 12-19.
27. Chandrasekaran M, Venkatesalu V. Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. J Ethno pharmacol 2004; 91: 105-108.
28. Meyer BN, Ferrigni NA, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. J. Med. Plants Res. 1982; 45:31-34.
29. Naz R, Ayub H, Nawaz S, Islam ZU, Yasmin T, Bano A, et al. Antimicrobial activity, toxicity and ant-iinflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. BMC Complement Altern Med 2017;17:302. doi:10.1186/s12906-017-1815-z.

30. Gould IM, Bal AM. New antibiotic agents in the pipeline and how they can overcome microbial resistance. *Virulence* 2013; 4(2):185–191.

31. Sengupta S, Chattopadhyay MK, Grossart HP. The multifaceted roles of antibiotics and antibiotic resistance in nature. Front Microbiol 2013;4:47.

32. Read AF, Woods RJ. Antibiotic resistance management. Evol Med Public Health 2014;2014(1):147.

33. Lushniak BD. Antibiotic resistance: a public health crisis. *Public Health Rep* 2014;129(4):314-316.

34. Piddock LJ. The crisis of no new antibiotics-what is the way forward? Lancet Infect Dis 2012;12(3):249-253.

35. Nasreen S, Ahmed T. Food Adulteration and Consumer Awareness in Dhaka City, 1995-2011. J Health, Population and Nutrition. 2014; 32(3):452-464.

36. Ghosh A, Das BK, Roy A, Mandal B, Chandra G. Antibacterial activity of some medicinal plant extracts. J Nat Med 2008; 62(2):259-62.

37. Koduru S, Grierson DS, Afolayan AJ. Antimicrobial activity of *Solanum aculeastrum*. Pharm Biol 2006; 44:283-286.

38. Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO. Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity. J Ethnopharmacol 2001; 78:119-127.

39. Londonkar RL, Kattegouga UM, Shivsharanappa K, Hanchinalmath JV. J Pharm Res 2013; 6:280-283.

40. Kuete V. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Med. 2010;76:1479–91.

41. Islam R, Rahman MS and Rahman SM. GC-MS analysis and antibacterial activity of *Cuscuta reflexa* against bacterial pathogens. Asian Pac J Trop Dis 2015; 5(5): 399-403.

42. Alim S, Bairagi N, Shahriyar S, Kabir MM, Rahman MH. *In vitro* antibacterial potential of *Bixa orellana* L. against some pathogenic bacteria and comparative investigation on some standard antibiotics. J Pharmacogn Phytochem 2016; 5(2): 178-181.