Abstract
Background: Traditional systems of medicine are often a valuable source of novel antimicrobials. Dima Hasao Hill district is endowed with rich cultures of traditional system of medicine and this study is the first of its kind from the area. The aim of the study is to assess the antimicrobial efficacy of *Trema orientalis* Blume (Ulmaceae) on six selected bacterial strains. The minimum inhibitory concentrations were determined with the aqueous extract to validate the application of the plant species in traditional medicine.

Methods: Plant materials were collected after prior informed consent and processed using standard herbarium technique. Antimicrobial activity was determined by Kirby-Bauer Agar Disc Diffusion method with slight procedural modifications. Minimum inhibitory concentration of the aqueous extract was determined with standard antibiotics as positive control.

Result: The selected bacterial strains were highly susceptible to the test material. Aqueous extracts showed fairly good activity. The zones of inhibition of all the test materials ranged from 11 to 15 mm. MIC of aqueous extract showed inhibition of bacterial growth at a concentration as low as 0.625 mg/ml.

Conclusion: *T. orientalis* is a potentially good source of antibacterial agent. The efficacy against the selected bacterial strains and the resultant MIC values corroborates with its application in traditional medicine.

Keywords: Antimicrobial; Dima Hasao Hill district; *Trema orientalis*; plant extracts.

Introduction
According to the World Health Organization (WHO), infectious diseases are the primary cause of deaths worldwide and they account for more than 50% of the death in tropical countries (1). To combat such diseases, a number of antibiotics have been produced by pharmacological industries worldwide, but the resistance of microbes has also increased in parallel. Further, bacterial strains are becoming increasingly resistant to most of the antibiotics available in the market. This has resulted in multiple drug resistant microbial strains, and to combat them, copious number of synthetic drugs especially in the developing countries (2). Moreover, some antibiotics have serious undesirable side effects that limit their application.

There is a resurgence of interest in herbal medicines due to the increased awareness of the limitations of synthetic drugs and their undesirable side effects and the need to discover new molecular structures as lead compounds from plants. The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of...
given plant will reveal only a very narrow spectrum of its constituents (3). Historically, pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as a tool in discovering new biologically active molecules has been the most productive in the area of antibiotics (4, 5). Even now, contrary to common belief, drugs from higher plants continue to occupy an important niche in modern medicine. On a global basis, at least 130 drugs, all single chemical entities extracted from higher plants, or modified further synthetically, are currently in use, though some of them are now being made synthetically for economic reasons (6). Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is traditional medicines. Systematic screening of them may result in the discovery of novel effective compounds (7).

Dima Hasao Hill district, a small district of Assam, North-East India, located between 92º37’ E - 93º17’E longitudes and 23º30’N - 25º47’N latitudes, lies in one of the world’s 12 mega biodiversity hotspot regions. It is a living anthropological museum of many ethnic tribes, such as Dimasa, Zeme-Naga, Hmar, Kuki, Biate, Hrangkhol, Khelma, Jaintia, Karbi, Vaiphei etc., each with their own unique cultures and traditional system of healing. The small hill district has a total population of 1, 86,189 with a density of 38 persons per square kilometer, the lowest in the state of Assam (2001 census). The tribal villagers have considerable knowledge on the use of both conventional and non-conventional plants for curing many common as well as severe ailments. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency (8-12). Much work has already been done in India also (13-19) but very few reports exist from North-East India and such works have never been taken up from Dima Hasao Hill district of Assam.

A perusal of the available literature reveals that although reports on the application of plants from the northeastern region of India has exceeded 1350 species with ethnomedicinal uses, 665 as food plants and 899 species for miscellaneous uses (20), the small hill district still remains virtually unexplored except some sporadic reports by Tamuli et al., Sajem et al., and Rout et al (21-26). Set in this backdrop, Trema orientalis Blume., (Fig. 1), an evergreen tree, commonly found in the district, has been selected for antimicrobial screening (Table 1). The present paper describes the antibacterial activity of the bark extracts of Trema orientalis Blume., against six different bacterial strains viz., Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Bacillus subtilis and Escherichia coli.

Methodology

**Plant material:** The plant species (Fig. 1) was selected on the basis of its reported use against cuts and infected wounds by the Hmar and Zeme Naga tribes (Table 1) from the study area. Plant materials were collected from the traditional healers after prior informed consent. The species is identified using relevant literature (27-29) and

![Fig. 1. Trema orientalis plant used by the Hmar and Zeme tribes of North Cachar Hills district of Assam for the treatment of infected cuts and wounds.](image)

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Preparation of Extracts: Barks of healthy plants were collected from the study sites, washed thoroughly in tap water and dried in a dark room at normal temperature for 15 days. They were then grounded to fine powder using an electric blender. The powdered samples were then extracted following standard procedures with slight modification (32). An amount of 5g of powdered material were soaked separately in 40 ml each of four different solvents namely distilled water, ethanol, methanol and acetone by keeping in a shaker for 3 days. The extracts were filtered with Whatman filter paper no.1 and reduced to 10 percent of their original volume by concentrating in vacuum using a rotary evaporator.

Inoculums: The test microorganisms namely, Klebsiella pneumoniae ATCC-13588, Pseudomonas aeruginosa ATCC-1037, Proteus vulgaris ATCC-128, Staphylococcus aureus ATCC-0016, Bacillus subtilis ATCC-9372 and Escherichia coli ATCC-0127, were obtained from the Department of Biotechnology, Assam University, Silchar. The organisms were inoculated in to Mueller Hinton broth and incubated at 37°C overnight to bring them into their mid-logarithmic phases of growth. The bacterial cells were harvested by centrifuging at 500g for 15 minutes. The pellets formed were washed twice with phosphate buffer saline (PBS) and the cells were counted by a haemocytometer (33). The bacterial cells were then diluted at approximately 10^5 CFU (colony forming unit) per milliliter before use (33).

Determination of antibacterial activities: Determination of the antibacterial activity of the extracts was performed by Kirby-Bauer Agar Disc Diffusion method with slight procedural

<table>
<thead>
<tr>
<th>Botanical Name</th>
<th>Family</th>
<th>Local Name</th>
<th>Part Used</th>
<th>Method of use</th>
<th>Other use</th>
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<tbody>
<tr>
<td>Trema orientalis Blume</td>
<td>Ulmaceae</td>
<td>Hatou (Hmar)</td>
<td>Barks</td>
<td>Fresh barks are collected, washed in clean water and then pounded to pulp. It is then applied as a poultice in the affected area. Dressings are changed everyday till the wound is cured. The powder is pounded to a pulp with potassium permanganate (KMnO₄) and other ingredients using traditional local methods. The gun powder which is used for hunting is killed by the traditional healer.</td>
<td>in consultation with the Botanical Survey of India, BSI/APC (ARUN Herbarium,) Itanagar and BSI (Kanjilal Herbarium), Eastern circle, Shillong. The voucher specimen has been processed through standard herbarium techniques (30-31) and submitted in the Department of Ecology and Environmental Science, Assam University, Silchar, Assam, India.</td>
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<td>(Collector’s Initial/Herbarium Voucher No)</td>
<td></td>
<td>Kedubang (Zeme)</td>
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modifications (34). The agar plates were prepared by pouring 15 ml of molten Mueller Hinton agar media into sterile petriplates. The plates were allowed to solidify for 5 minutes and the agar medium was inoculated with test microorganisms by pour plate method. Discs (5mm diameter) were punched in Whatman number 1 filter paper. The dried and sterilized disc was then impregnated with known amount of the plant extract (50mg/ disc). The loaded disc was placed on the surface of the medium and the compound was allowed to diffuse for five minutes. The plates were then kept for incubation at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of zone of inhibition of the respective extracts with the help of a transparent ruler in millimeter (Table 1).

**Minimum Inhibitory Concentration (MIC):**
Minimum inhibitory concentration of the aqueous extract was determined as described by Kabir et al (35). The test was performed by serially diluting the extracts to various concentrations ranging from 10 mg/l to 0.02 mg/l. Each volume of each extract and nutrient broth were mixed in a test tube and the inoculum size was adjusted as to deliver a final inoculum of approximately 10^9 CFU per ml. Triplicates were maintained along with two control tubes for each test batch-tubes containing the growth medium, physiological saline and the inoculum (organism control) and tubes containing extract and the growth medium without inoculum (antibiotic control). The tubes were inoculated at 37°C for 24 hours. The lowest concentration of extract that produced no visible bacterial growth (no turbidity when compared with control tubes was regarded as MIC. Minimum inhibitory concentrations of standard antibiotics against the bacterial strains taken were also determined as positive control.

**Results and Discussion**
The profile of the plant used in this study is shown in Table-1. The Table-2 demonstrates the antibacterial activity of the plant extracts in different solvents. The study showed that all the bacterial strains used in the present study were highly susceptible to the test material. Ethanolic and methanolic extracts against S. aureus showed maximum activity against the microorganisms studied. The zones of inhibition of all the test materials against the gram positive bacteria ranged from 12 to 15 mm and that of the Gram negative bacteria ranged from 11 to 14 mm showing that Gram negative bacteria is marginally more resistant in agreement with previous reports (36-40).

Table-3 shows that the aqueous extract of the test material presented similar MIC’s against *Pseudomonas aeruginosa* and *Proteus vulgaris* at a concentration of 1.25 mg/ml. *S. aureus* and *E.coli* were found to be inhibited at an MIC of 2.5 mg/ml, Lowest MIC (0.625 mg/ml) was observed against *K. pneumoniae* and *B. subtilis*. However no significant trend was noticeable for the different solvents.

The present finding on the antibacterial activity of the present test material against different strains validates the traditional use of these species by the two tribes against infected wounds. Pertinent here is to mention that the traditional use of the plants by the tribes always involve aqueous extracts. The study showed that aqueous extracts also showed fairly good activity against the bacterial strains. Considering the inherent toxicity of the non aqueous solvents, the aqueous extract holds significant promise for useful phytochemicals. Infections caused by *P.aeruginosa* especially those with multi drug resistance are among the most difficult to treat with conventional drugs (39). In the present study, growth of *P.aeruginosa* was inhibited by the aqueous extract of the test material at a concentration as low as 1.25 mg/ml (Table 3).

Choudhury and Islam (40) worked on the antimicrobial efficacy of ethyl acetate, n-hexane and methanolic extracts of the root of *T. orientalis* and found that no extract was active against *Klebsiella sp*, *P. aeruginosa*, *B. subtilis* and *S. aureus* even at a dose of 500 mg/disc. Only methanolic extract showed significant activity against *E. coli*. Our present study on the aqueous

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Current Trends in Biotechnology and Pharmacy
Vol. 6 (4) 464-471 October 2012, ISSN 0973-8916 (Print), 2230-7303 (Online)

extract of bark of the same plant species, on the other hand is in contrast to the above report. Our test material showed significant activity against all the bacterial strains at a dose of 50 mg/disc giving an inhibition zone ranging from 11 to 14 mm against \textit{K. pneumoniae}, 12 to 13 mm against \textit{P. aeruginosa}, 12 to 14 mm against \textit{B. subtilis}, and a the best zones ranging from 14 to 15 mm against \textit{S. aureus} (Table-2). MIC of aqueous extract further revealed that the bacterial strains are inhibited at a concentration as low as 0.625 mg/ml (Table-3).

**Conclusion**
The results of the present study have shown that \textit{T. orientalis} Blume is a potentially good source of antibacterial agent. Significant efficacy against the selected bacterial strains and the resultant MIC values of aqueous extract corroborates the traditional medicinal application of the plant species. The study area with its vast ethnobotanical wealth deserves extensive exploration of their potentials in the discovery of newer active principles which could lead to the development of newer and safer drugs.

**Acknowledgements**
Thanks are due to Botanical Survey of India, Shillong and Itanagar for identification of the specimens. We thank Dr.P.K.Hajra, former Director, Botanical Survey of India for his assistance in the identification of the species collected. We thank all the informants who contributed to this study with their valuable traditional knowledge. Acknowledgement is due

| Table 2. Antibacterial activity of \textit{Trema orientalis} Blume in different solvent extracts. |
|---|---|---|---|---|---|---|
| Species | Part used | Concentration | Solvent | Zone of Inhibition (mm) |
| | | | | Ec | Kp | Pa | Pv | Sa | Bs |
| \textit{T. orientalis} Blume. | Bark | 50mg/disc | D | 11 | 12 | 13 | 12 | 14 | 12 |
| | | | E | 13 | 14 | 13 | 12 | 15 | 13 |
| | | | M | 12 | 12 | 12 | 14 | 15 | 14 |
| | | | A | 14 | 15 | 13 | 14 | 11 | 12 |

D - Distilled water; E - Ethanol; M - Methanol; A - Acetone. 

| Table 3. Minimum inhibitory concentration of aqueous extract of \textit{Trema orientalis} Blume. |
|---|---|---|
| Bacterial strains | Minimum Inhibitory Concentration (mg/l) |
| Aqueous extract of \textit{Trema orientalis} Blume | Antibiotics |
| \textit{Klebsiella pneumoniae} | 0.625 | Ciprofloxacin | 0.21 |
| \textit{Pseudomonas aeruginosa} | 1.25 | Ciprofloxacin | 0.12 |
| \textit{Proteus vulgaris} | 1.25 | Cefpodoxim | 2.56 |
| \textit{Staphylococcus aureus} | 2.5 | Vancomycin | 1.36 |
| \textit{Bacillus subtilis} | 0.625 | Ampicillin | 0.007 |
| \textit{Escherichia coli} | 2.5 | Ciprofloxacin | 0.008 |

Antibacterial efficacy of \textit{Trema orientalis}
to the Department of Biotechnology, Assam University for all the laboratory facilities and to the Department of Botany, Haflong Government College for logistic support.

References


**Antibacterial efficacy of Trema orientalis**


