ETHANOLIC EXTRACT OF MILLETIA FERRUGINEA INHIBITS GROWTH OF SHIGELLA SPECIES

Sayani Marick¹, Debasmita Chatterjee², Manash Kumar Choudhury³ and Satadal Das*⁴

¹Department of Microbiology, Techno India University, Salt Lake City, Kolkata, India.
²Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata, India.
³Department of Chemistry, the School of Postgraduate Studies, Dilla University, Dilla, Ethiopia.
⁴Department of Microbiology, Peerless Hospital and B. K. Roy Research Centre, Kolkata, India.

ABSTRACT

The plant *Milletia ferruginea* is a large tree and is widely distributed all over Ethiopia. It is commonly known as ‘Berebera’. Literature survey revealed that this plant has wide application against skin infection and is also effective against storage insect pests. This study was done to evaluate the antimicrobial activity of this African medicinal plant against *Shigella* species. Seeds of the plant *M. ferruginea* was extracted using absolute alcohol and studied for their antimicrobial activities against *Shigella* species which cause bacillary dysentery in human beings, particularly in children with high mortality and morbidity. The antimicrobial activities were tested using agar well diffusion and 96 well microtitre plate method. Extracts of the plant showed antimicrobial activities against *Shigella* spp. The seed extract inhibited the growth of *Shigella boydii, S. dysenteriae MDR, S. flexneri 2a MDR, S. flexneri 4a* showing the MIC value within the range of 0.32mg/mL to 2.6mg/mL. This finding indicates its possible future use as antimicrobial agent against *Shigella* sp.

KEYWORDS: *Milletia ferruginea. Shigella* sp., antibacterial activity, minimum inhibitory concentration.
INTRODUCTION
Nature is a never-ending source of novel antimicrobials and continuous research is going on to explore this. Literature survey revealed the medicinal benefits of plants against various ailments. Parts of plants such as its leaf, bark, roots and sometimes the whole plant extracts are used against various diseases.\(^1\) As plants are a rich source of antimicrobial agents, therefore, it is an interesting area of research for the microbiologists as lots of phytochemicals find their way to the repertoire of antimicrobial drugs to be prescribed by the physicians.\(^2\) Plants contain various biologically active compounds which are also identified as its secondary metabolites such as phenols, flavonoids, tannins, saponins and alkaloids particularly in young plants.\(^3\) 

*M. ferruginea* is one of the useful endemic tree species of Ethiopia. It has great potential for agro forestry. It belongs to the family Fabaceae (Leguminosae), sub family Papilionadeae. This plant has two subspecies, namely *ferruginea* and *darassana*.\(^4\) It is a nitrogen fixing leguminous tree and is also famous for its multipurpose utility. The wood of this plant is used to fire wood, for constructing house, the flowers serve as feed for bees. Its leaves, shoots and flowers are used as fodder for ruminants. This plant is also used to make household utensils, as erosion control, for fencing and as a shade tree in coffee growing areas. It is also used as a decorative plant that can be planted along with other plants.\(^5\) Only a few studies are available regarding the antimicrobial activities of *M. ferruginea*. A study indicated antibacterial activity of the extract of the leaf of this plant against *Streptococcus pyogenes* causing wound infection.\(^6\) 

Now-a-days resistance to commercially available antibiotic is becoming a matter of great concern to the clinicians. Shigellosis is a highly prevalent disease among children in all developing countries and about 85% of the *Shigella* sp., which are isolated from stools and rectal swab samples shows multi antibiotic resistance. They showed high MIC values against frequently used antimicrobials like ampicillin, tetracycline, nalidixic acid and co-trimoxazole.\(^7\) Therefore, we must aim to curb down this growing antibiotic resistance among *Shigella* spp. In the present study we have evaluated the antibacterial activity of *M. ferruginea* against few *Shigella* sp. isolated from bacillary dysentery cases and two of them were MDR bacteria.
MATERIALS AND METHODS

Collection and processing of the plant material (This was done mainly by Author MKC)
The mature seeds of *M. ferruginea* subspecies *darassana* were collected from a place near Dilla University campus, Dilla, Ethiopia in the months of December and January authenticated at the National Herbarium, Addis Ababa University. A voucher specimen (S-108) of the plant sample was deposited at the department of biology, Addis Ababa University, Ethiopia. The seeds were powdered using a grinding machine. Other plant materials were washed in tap water, dried and then powdered using wooden made pestle and mortar.

**Preparation of plant extracts**
5gm of powdered sample of the plant were then extracted using 50ml absolute (99.8%) ethanol (1:10) for 72 hours. The mixture was periodically shaken. After 72 hours the extract was filtered using Whatman filter paper No. 1. The solvent was evaporated using water bath set at temperature 80°C. After evaporation of the solvent the active components were dried (at 4°C) for future analysis. The crude contents were dissolved in DMSO (dimethyl sulfoxide) for further antibacterial assays.\[8\]

**Microorganisms used**
WHO EQAS strains of *Shigella* spp. namely *S.flexneri* type 4a, *S. boydii*, were used in this study along with two multidrug resistant strains of *S.flexneri* type 2a MDR and *S. dysenteriae* MDR which were collected from National Institute of Cholera and Enteric Diseases, Kolkata, India.

**Antibacterial assay by agar diffusion**
Bacterial suspension equivalent to 0.5 MacFarland opacity was used and a uniform lawn culture was done with sterile cotton swab stick on Mueller Hinton agar. After soaking the plates for about 15 minutes, sterile discs made of Whatman filter paper No.1 of 6mm diameter were placed upon bacterial lawn culture and were impregnated with 20µL extracts (40mg/mL) and solvent control (DMSO). All the plates were kept in incubation at 37°C for next 16-18 hours. After the incubation period the sensitivity zone if any was observed and the diameters were recorded.\[9\] All the assays were done in triplicate.
Antibacterial assay by determining minimum inhibitory concentration
MIC assay was then conducted in a 96 well microtitre plate. 0.5 MacFarland opacity bacterial suspensions were used for the assay. The extracts (40mg/ml) were serially diluted with Mueller Hinton broth in the 96 well microtitre plate. 10µL of bacterial suspensions were inoculated in each concentration of the extract. The plate was incubated for 24 hours at 37°C and the growth of the bacterium was evaluated by comparing the absorbance (OD) of each well before and after the incubation by using a 96 well plate reader (Erba Lisa Scan II Transasia Mannheim, Germany). [9]

RESULTS AND DISCUSSION
In agar disc diffusion assay sensitivity zones were found with three species out of the four tested species (Table 1). Control antibiotic (ciprofloxacin) also showed sensitivity zones. MIC values against the different bacteria were also estimated (Fig. 1).

Table 1: The table represents the zone of inhibition (mm) of extract of M. ferruginea against Shigella spp.

<table>
<thead>
<tr>
<th>SL.NO.</th>
<th>Microorganisms used</th>
<th>ZI (mm) OF M. ferruginea</th>
<th>ZI (mm) OF antibiotic control (Ciprofloxacin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>S. boydii</td>
<td>11</td>
<td>33</td>
</tr>
<tr>
<td>2.</td>
<td>S. flexneri 4a</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td>3.</td>
<td>S. flexneri 2a MDR</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>4.</td>
<td>S. dysenteriae MDR</td>
<td>No zone</td>
<td>17</td>
</tr>
</tbody>
</table>

Fig: 1 The bar diagram represents the mean ± SD of minimum inhibitory concentration (mg/mL) of the extract against different Shigella spp.
Ethnobotanical investigations are offering us important clues in the identification and development of traditionally useful medicinal plants into modern drugs. This field of science is contributing a lot in modern studies and detailed researches on plant materials are leading to the invention of new drugs.\cite{6} Toxicity of the plant was studied earlier. \textit{In vivo} and \textit{in vitro} study on toxicity of the extract of \textit{M. ferruginea darassana} against adult male and female three host tick named \textit{Amblyomma variegatum} was studied. The root and bark of the roots showed toxicity whereas the leaves did not display any toxicity.\cite{10} Here in this study, the antimicrobial activity of crude extracts of seeds of \textit{M. ferruginea} was explored against four \textit{Shigella} strains namely \textit{S. boydii}, \textit{S. dysenteriae} MDR, \textit{S. flexneri} 2a MDR, \textit{S. flexneri} 4a.

The result of the agar disc diffusion assay indicated the presence of anti-shigellosis activity of the plant extract (Table 1). The ethanolic extract showed effective antimicrobial activity against all the four strains of \textit{Shigella} as observed after the MIC analysis (Fig. 1).

In our study the MIC of the plant extract against \textit{S. boydii} was found to be the lowest, 0.328mg/ml. Similarly the MIC value obtained against \textit{S. dysenter}y MDR was 2.625mg/ml was found to be the highest (Fig. 1). The two strains used in this experiment were resistant to many antibiotics. The MIC of the plant extract against \textit{S. flexneri} 2a MDR and \textit{S. flexneri} 4a were 0.656 mg/ml and 1.3125mg/ml respectively (Fig: 1). Study showed that many strains of \textit{Shigella} are showing multidrug resistance\cite{11}, thus this study may help in finding new antimicrobials from this plant which may act against them.

\textbf{CONCLUSION}

This study revealed effective antimicrobial activity of ethanolic extract of the seeds of African medicinal plant \textit{M. ferruginea} against \textit{Shigella} spp.

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REFERENCES