Introduction

Bacterial pathogens have evolved multiple defense mechanisms against antimicrobial agents and resistance to old and newly produced drugs [1]. Recently, the use of drugs and dietary complements, extracted from plants, has been accelerated [2]. In fact, according to the World Health Organization (WHO), almost 25% of modern drugs used in the United States have been extracted from plants [3].

The *Mangle negro* is a species of flowering plants in the acanthus family, Acanthaceae. *M. negro* is a subtropical wooden shrub that grows in salt lagoon. *M. negro* is very hard, adapted to harsh environments where water and salinity levels oscillate [4]. Leaves are 1 to 5 inches long, elliptical, contrary, big, leathern, dark green, glabrous (smooth) above and grayish with a tight felt-like pubescence beneath. The glands on the underside secrete pubescence. The glands on the underside secrete (smooth) above and grayish with a tight felt-like pubescence beneath. The glands on the underside secrete pubescence. The glands on the underside secrete pubescence beneath. 

**Keywords:**
- Bacillaceae
- Enterobacteriaceae
- Mangle negro

Materials and Methods

**Plant extraction:** Fresh cultivated *S. typhimurium* ATCC 14028, *P. vulgaris* ATCC 8427, *E. coli* ATCC 25922, *B. cereus* ATCC 14579 and *B. subtilis* ATCC 23857 colonies were suspended in 5 mL of 0.85% normal saline (DEFCO Laboratories, USA). Infectious diseases are caused by pathogenic microorganisms, such as bacteria, viruses or fungi; the diseases can be spread, directly or indirectly, from one person to another. Zoonotic diseases are infectious diseases of animals that can cause disease when transmitted to humans. Inappropriate use of antibiotics has helped create strains of bacterial disease resistant to treatment with different types of antibiotic medications [1].

In this study, the antibacterial activity of *M. negro* ethanolic and aqueous extracts were evaluated, through disk agar diffusion method, extract on medium surface method and microdilution method, against *Salmonella typhimurium* ATCC 14028, *Proteus vulgaris* ATCC 8427, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 14579 and *Bacillus subtilis* ATCC 23857 in vitro.

**Materials and Methods**

This experimental study was conducted at Industrial Microbiology Laboratory, Department of Food Sciences and Technology, Ferdowsi University of Mashhad in 2014. The *M. negro* was collected from countryside of Bandar-e Mahshahr (Khuzestan Province, Iran).

**Abstract**

**Background:** In this study, the antibacterial activity of *Mangle negro* extract against selected pathogens from Enterobacteriaceae and Bacillaceae was evaluated.

**Materials and Methods:** This experimental study after collection and preparation of aqueous and ethanolic extracts of *M. negro*, their effects against human pathogen microorganism were determined.

**Results:** The ethanolic and aqueous extracts inhibited the growth of all the organisms tested. The minimum inhibitory concentration (MIC) of the extracts ranged between 4 mg/mL and 64 mg/mL.

**Conclusion:** The study demonstrated that the ethanolic leaf extract of *M. negro* hold an excellent potential as an antibacterial agent.
in triplicates and then they were placed in an incubator at 37°C for 18-24 h. The color change was then assessed visually and the lowest concentration at which the color change occurred was taken as the MIC value [8].

Minimum lethal concentration (MLC): The MLC was determined through a series of steps, undertaken after a MIC test has been completed [9]. The employed software was SPSS-18 (USA, II, Chicago, SPSS Inc). Differences at p<0.05 were considered to be significant.

Results

Antimicrobial effects of ethanolic and aqueous extracts of *M. negro*, by the agar diffusion method are presented in table 1. The zone of inhibition in the ethanolic extract varied from 8.5 mm for *S. typhimurium* to 24 mm for *B. subtilis* and from 7 mm for *S. typhimurium* to 19 mm for *B. subtilis* in the aqueous extract. It could be seen the both ethanolic and aqueous extracts inhibited the growth of all the test organisms. The MIC and MLC results of ethanolic and aqueous extracts of *M. negro* are presented in table 2. The MIC of ethanolic extract of *M. negro* for *S. typhimurium*, *P. vulgaris*, *E. coli*, *B. cereus* and *B. subtilis* were 32, 8, 16, 4 and 8 mg/mL, respectively. The MIC of the aqueous extract of *M. negro* for *S. typhimurium*, *P. vulgaris*, *E. coli*, *B. cereus* and *B. subtilis* were 64, 16, 32, 8 and 8 mg/mL, respectively.

The MLC of ethanolic extract of *M. negro* for *S. typhimurium*, *P. vulgaris*, *E. coli*, *B. cereus* and *B. subtilis* were 64, 16, 32, 8 and 4 mg/mL, respectively. The MLC of the aqueous extract of *M. negro* for *S. typhimurium*, *P. vulgaris*, *E. coli*, *B. cereus* and *B. subtilis* were 128, 32, 64, 16 and 8 mg/mL, respectively.

Discussion

Results obtained from the present study revealed the possess potential antibacterial activity of them two tested extracts against *S. typhimurium*, *P. vulgaris*, *E. coli*, *B. cereus* and *B. subtilis*. The highest antibacterial activity of 24 mm was in *B. subtilis* while the least activity was recorded in *S. typhimurium* measured 19.30 mm. Tested by the disc diffusion method, the aqueous leaf extracts of *M. negro* showed significant activity against *S. typhimurium*, *P. vulgaris* and *E. coli* around 16.5 mm.

On the basis of the above results, it showed that ethanolic extract of *M. negro* exhibited a greater inhibition compared with aqueous extract. Owojab and Omogbai reported that most of the antimicrobial active compounds were soluble in a polar solvent like ethanol instead of water [10]. This result is comparable to the study by Nwinyi et al. using ethanolic extract of *Ocimum gratissimum* that showed effective antibacterial activity on *E. coli* and *Staphylococcus aureus* [11]. Kareem et al. studied the antimicrobial effect of ethanolic, aqueous and chloroform extracts of leaf and latex of *Calotropis procera* on 6 bacteria, 4 fungi and yeast, using agar well diffusion and paper disk methods. The results revealed that the ethanolic was the best extraction solvent for antimicrobial properties of leaf and latex of *C. procera*, followed in order by chloroform and aqueous extracts [12].

**Table 1.** Average diameter (mm) of microbial free zone area of aqueous and ethanolic Mangle negro extracts concentrations on Salmonella typhimurium, Proteus vulgaris, Escherichia coli, Bacillus cereus and Bacillus subtilis (disk agar diffusion method)

<table>
<thead>
<tr>
<th>Type of extract</th>
<th>Microorganism</th>
<th>The concentration of Mangle negro extracts (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td><em>S. typhimurium</em></td>
<td>7.0±0.50*  12.0±0.50*  19.90±0.28c  24.00±0.58c</td>
</tr>
<tr>
<td></td>
<td><em>P. vulgaris</em></td>
<td>8.3±0.50*  11.4±0.54a  14.80±0.54a  17.00±0.50d</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>10.2±0.58b  15.50±0.55c  19.10±0.50c  23.00±0.58d</td>
</tr>
<tr>
<td></td>
<td><em>B. cereus</em></td>
<td>13.6±0.58a  15.80±0.50c  19.00±0.54d  22.00±0.50d</td>
</tr>
<tr>
<td></td>
<td><em>B. subtilis</em></td>
<td>11.1±0.57a  15.50±0.55c  19.90±0.50c  24.00±0.58d</td>
</tr>
<tr>
<td></td>
<td><em>S. typhimurium</em></td>
<td>8.5±0.58b  12.0±0.57a  16.40±0.55c  20.10±0.28d</td>
</tr>
<tr>
<td></td>
<td><em>P. vulgaris</em></td>
<td>9.0±0.58a  12.0±0.58a  16.20±0.50c  19.90±0.50d</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>9.0±0.54a  12.0±0.58a  16.20±0.50c  19.90±0.50d</td>
</tr>
<tr>
<td></td>
<td><em>B. cereus</em></td>
<td>10.7±0.58a  14.3±0.50b  17.50±0.28c  21.90±0.57c</td>
</tr>
<tr>
<td></td>
<td><em>B. subtilis</em></td>
<td>12.0±0.58a  15.1±0.50a  19.90±0.28c  24.00±0.58d</td>
</tr>
</tbody>
</table>

"Values are means ±standard deviations, N=3.

**Table 2.** Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of aqueous and ethanolic extract of Mangle negro on Salmonella typhimurium, Proteus vulgaris, Escherichia coli, Bacillus cereus and Bacillus subtilis

<table>
<thead>
<tr>
<th>Type of extract</th>
<th>Bacteria species</th>
<th>MIC (mg/mL)</th>
<th>MLC (mg/mL)</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td><em>S. typhimurium</em></td>
<td>64</td>
<td>128</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>P. vulgaris</em></td>
<td>16</td>
<td>32</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>32</td>
<td>64</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>B. cereus</em></td>
<td>8</td>
<td>16</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>B. subtilis</em></td>
<td>8</td>
<td>8</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>S. typhimurium</em></td>
<td>32</td>
<td>64</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>P. vulgaris</em></td>
<td>8</td>
<td>16</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ethanolic</td>
<td><em>E. coli</em></td>
<td>16</td>
<td>32</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>B. cereus</em></td>
<td>4</td>
<td>8</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>B. subtilis</em></td>
<td>4</td>
<td>4</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Grow, –Not grow, N=3.
The minimum inhibitory concentration values of the plant extracts against the test organisms showed that the bacteria varied widely in the degree of their susceptibility to antibacterial agents. This agrees with the report that antimicrobial agents with low activity against an organism have high MIC while a highly antimicrobial agent has a low MIC [13]. The results indicated that the MLC of the extracts were higher than the MIC. This observation, therefore, suggests that the antimicrobial substances contained in the extracts were fungistatic at lower concentrations while becoming fungicidal at higher concentrations of the extracts. Similar observations have been reported by Tabatabaei-Yazdi et al. [14]. Mann et al. investigated the antibacterial effects of ethyl acetate, chloroform, ethanolic, methanolic and aqueous root extracts of Anogeissus leiocarpus and Terminalia avicennioides in vitro for antifungal activities against Aspergillus fumigatus, Aspergillus niger and Penicillium species. They reported that ethanolic extracts of the two plant roots were more effective than the methanolic, chloroform or aqueous extracts against all the test fungi pathogens [13]. In another study, Ekpo and Etim reported that ethanolic and aqueous extracts of Sida acuta were effective against both Gram positive (Staphylococcus aureus, B. subtilis) and Gram negative bacteria (Pseudomonas aeruginosa, E. coli) and the antibacterial effect was greater against Gram positive bacteria than against Gram negative bacteria, showing similar results to those of this study [15]. It was also reported by El-Mahmod et al. that the antibacterial screening of the aqueous and ethanolic extracts of the various plant materials were carried out against pathogenic bacteria including P. aeruginosa, Klebsiella pneumonia, E. coli, S. aerius and Shigella dysenteriae. Ethanolic extracts were more potent than aqueous extracts and the activity intensity was concentration dependent. The Gram positive bacteria were more sensitive to the ethanolic extracts of both plants [16].

The results seem to legitimize the continued use of the extracts in the order of microbial infections. The inhibition of growth of the test organisms, that are known to cause nosocomial infections and displaying multidrug resistant to most antibiotics and non-antibiotic antimicrobial agents, legitimize the continued application of these plants in public and traditional medical practice. Studies should therefore be done in order to know the active phytochemical constituent of the extracts and appraise their effectiveness in vitro so that they can be combined and joinery production begins in serious.

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Authors’ Contributions
All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest
The authors declare no conflict of interest.

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References

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