INTRODUCTION

*Moringa oleifera* Lam. (Moringaceae), commonly referred to as “drumstick” is a small- to medium-sized tree, distributed in many countries of the tropics and subtropics. Several parts of this specie have nutritional, therapeutic and prophylactic properties and are used in traditional medicine in treating all sorts of diseases including diarrhea, one of the most common cause of morbidity and mortality worldwide (Fahey, 2005; Goyal et al., 2007; Arora et al., 2013; Gopalakrishnan et al., 2016). It is therefore important to evaluate available natural alternatives to currently used as anti-diarrheal drugs, which are not always free from adverse effects.

Literature suggested that some parts of this specie had potential as an anti-diarrheal agent. Some studies about these anti-diarrheal properties were carried out in animal models. Significant reduction in the severity and frequency of diarrhea were observed with aqueous, methanolic, ethanolic and hydroalcoholic leaves extract of *M. oleifera*, as well as methanolic root extract (Saralaya et al., 2010; Lakshminarayana et al., 2011; Choudhury et al., 2013; Misra et al., 2014).

The ethanolic, methanolic, chloroform, aqueous extract of leaves, seeds, root bark, fruits, flowers were also investigated against gram positive and gram negative bacterial and fungal pathogens, with variable antimicrobial activity depending on the solvent employed in extraction (Nikkon et al., 2003; Doughari et al., 2007; Rahman et al., 2009; Bukar et al., 2010; Vieira et al., 2010; Talreja, 2010; Sayeed et al., 2012; Oluduro, 2012; Kalpana et al., 2013; Zaffer et al., 2014; Fadeyi et al., 2015; Shailemo et al., 2016).

Even though the observed antimicrobial effects against *Bacillus* spp. there is no data about its activity against other spore-forming bacteria, like the genus *Clostridium* spp. Thus, the present study aims to evaluate the *in vitro* antibacterial activity of edible parts of *M. oleifera* against spore-forming bacteria associated with diarrhea such as *Clostridium difficile* and *Clostridium perfringens.*
MATERIALS AND METHODS

Plant material: The fresh leaves (green-stemmed leaves and red-stemmed leaves) and seeds of *M. oleifera* were donated by the company Agronature 2000 S.L., Cartagena, Murcia, Spain. Leaves were cleaned with a chlorine solution (150 ppm) and then dried with a laminar air flow. To obtain leaf powder and seed powder, fresh leaves and seeds were dried at 40°C for 7 days and then ground into a fine powder (Karthvy et al., 2009; Devendra et al., 2011).

Preparation of extract: Ethanolic, methanolic, aqueous and acetone extracts in several presentations of *M. oleifera* (fresh leaf, leaf powder, whole seed powder and seed husk powder) were obtained according to Karthy et al. (2009), Vijay and Samrot (2010), Onuoha and Alisa (2013) and Shailemo et al. (2016), with few modifications. The weighed amount of *M. oleifera* part (25 g of leaves or 40 g of seeds) were mixed with 100 mL extraction solvent and were macerated with intermittent shaking at room temperature (26°C) (Table 1). The percolates were filtered with filter paper (Whatman No. 1) and concentrated under vacuum at 40-60°C using arotary evaporator. The extracts were weighed, dissolved in the minimal volume of solvent and stored in a refrigerator at 4°C for antibacterial activity test.

Test bacterial strains and growth conditions: Typed strains of *Clostridium difficile* (CECT 531) and *Clostridium perfringens* (CECT 376) were obtained from Spanish Culture Collection Type at the University of Valencia, Spain. Test strains were routinely cultured on Brain Heart Infusion (BHI) agar (Oxoid) under anaerobic conditions (80% N₂, 10% CO₂ and 10% H₂) at 37°C in an anaerobic chamber (bioMérieux) for 24-48 h.

Screening extracts for antibacterial activity: To determine the susceptibility of the strains to *M. oleifera* extracts, broth microdilution assays were performed according to the Clinical and Laboratory Standards Institute methodology (CLSI, 2010) with few modifications. Briefly, a series of two-fold dilutions of each extract were made in a 96-well microtitre plate in BHI broth. Each dilution was performed in duplicate wells. Suspensions of tested organisms cultured on BHI agar during 24 h were adjusted to 0.5 McFarland (1.0×10⁸ CFU/mL) in 0.85% saline solution (bioMérieux) with the Densimat photometer (bioMérieux). Then it was inoculated in each well of microtitre plate to correspond to a final inoculum concentration of approximately 1.0×10⁷ CFU/mL. Growth was measured spectrophotometrically by optical density at 600 nm (OD600) with Microplate Reader (BioTek) at 48 h after anaerobic incubation at 37°C. The difference in OD600 measurements in the control samples between 0 and 48 h of incubation was defined as 100% growth. Differences in OD600 measurements at 0 and 48 h of incubation in the tested samples were calculated as percentages of the value obtained from the control. The Minimum Inhibitory Concentration (MIC) was determined as the lowest extract concentration resulting in a growth reduction at least of 0.1% compared to the respective control samples. The Minimum Bactericidal Concentration (MBC) was determined as the lowest concentration of extract resulting in a growth reduction >99.9%. Each plate tested was performed by duplicate in separated experiments.

Statistical analysis: The values of MIC and MBC obtained were calculated from the median values of percentages from, at least, three duplicates.

RESULTS AND DISCUSSION

Minimum inhibitory and bactericidal concentrations of the different extracts against tested pathogens are shown in Table 2 and 3, respectively.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Plant presentation</th>
<th>Extraction solvent</th>
<th>Sample</th>
<th>Maceration time</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green-stemmed leaf</td>
<td>Fresh</td>
<td>Acetone</td>
<td>25 g sample + 100 mL solvent</td>
<td>18 h</td>
<td>Acetone</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Cold water</td>
<td>25 g sample + 100 mL solvent</td>
<td>7 days</td>
<td>DMSO 3%</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Ethanol 95%</td>
<td>25 g sample + 100 mL solvent</td>
<td>7 days</td>
<td>Ethanol 95%</td>
</tr>
<tr>
<td>Red-stemmed leaf</td>
<td>Fresh</td>
<td>Ethanol 95%</td>
<td>25 g sample + 100 mL solvent</td>
<td>7 days</td>
<td>Ethanol 95%</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Ethanol 95%</td>
<td>25 g sample + 100 mL solvent</td>
<td>7 days</td>
<td>DMSO 3%</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Powder</td>
<td>25 g sample + 100 mL solvent</td>
<td>7 days</td>
<td>Ethanol 95%</td>
</tr>
<tr>
<td>Whole seed</td>
<td>Powder</td>
<td>40 g sample + 100 mL solvent</td>
<td>72 h</td>
<td>DMSO 3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>Water</td>
<td>40 g sample + 100 mL solvent</td>
<td>72 h</td>
<td>Ethanol 50%</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Ethanol 100%</td>
<td>40 g sample + 100 mL solvent</td>
<td>72 h</td>
<td>DMSO 30%</td>
</tr>
<tr>
<td>Seed husk powder</td>
<td>Powder</td>
<td>Methanol 99.9%</td>
<td>40 g sample + 100 mL solvent</td>
<td>72 h</td>
<td>DMSO 30%</td>
</tr>
</tbody>
</table>

Minimum inhibitory and bactericidal activities of ethanolic extract, for both leaves and seeds, was observed. It should be noted that red-stemmed leaves were exhibited more antimicrobial activity than green-stemmed leaves, showing lower MICs and MBCs. The acetone extract the fresh green-stemmed leaf was also shown to be active at lower concentrations than ethanolic extract. However, the aequous extract only showed antibacterial activity for whole seeds. Aqueous extract the fresh green-stemmed leaf was not active against *Clostridium* spp.
Bacillus cereus phytocomponents of another spore-forming genus, has been observed in strains tested in our study. The observed antimicrobial effect of extracts could be explained by the rich composition of important phytoconstituents of M. oleifera. Compounds such as 4′-(4′-O-acetyl-α-Lrhamnopyranosyloxy) benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate and 4-(α-Lrhamnopyranosyloxy) benzyl glucosinolate, as well as carotenoids, niaziridin and niazirin, have been attributed as the active antimicrobial principles of M. oleifera (Caceres et al, 1991; Karim and Azlan, 2012).

This chemical composition of the plant could be influenced by geographical location, altitude or temperature. For this reason, further studies are necessary for chemical characterization and extraction of the active principles and comprehension of the mechanism of antibacterial action of M. oleifera extracts.  

**CONCLUSION**

The extracts of the tested edible parts of M. oleifera showed varying degree of antibacterial activities against the tested bacterial species. Regardless extraction solvent, red-stemmed fresh leaves exhibited the greatest inhibitory effects. Whole seed showed more antibacterial activity than husk seed.

The ethanolic extract of leaves (especially from red-stemmed leaves) and seeds exhibited antibacterial activity, contrasting with the strains tested in our study. The observed antimicrobial effect of extracts could be explained by the rich composition of important phytoconstituents of M. oleifera. Compounds such as 4′-(4′-O-acetyl-α-Lrhamnopyranosyloxy) benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate and 4-(α-Lrhamnopyranosyloxy) benzyl glucosinolate, as well as carotenoids, niaziridin and niazirin, have been attributed as the active antimicrobial principles of M. oleifera (Caceres et al., 1991; Karim and Azlan, 2012).
with this organic solvent provided a considerable antibacterial activity.

The inhibitory effect of M. oleifera extracts observed against pathogenic Clostridium spp. can introduce the plant as a potential candidate for drug for the treatment of infection caused by these pathogens, as well as a sanitizer or preservative in foods against these foodborne microorganisms often implicated in the spoilage of foods and foodborne illnesses.

**CONFLICT OF INTEREST**

The authors declare no financial, commercial or academic conflicts of interest.

**REFERENCES**


