Chemical composition and antimicrobial activity of essential oil of fruits of morinda citrifolia linn
ABSTRACT

The fruit of the *Morinda citrifolia* species popularly known as “noni” has been widely used in folk medicine for diverse therapeutic purposes including in the treatment of infectious diseases. Given this, we tested the essential oil from *M. citrifolia* fruits obtained by hydrodistillation against fungi and bacteria. The oil was rich in short-chain fatty acids: octanoic acid (38.7%) and hexanoic acid (20.0%) were identified as the major constituents after analysis of oil by GC/MS. The oil showed antimicrobial activity against all the microorganisms tested, mainly against fungi *Candida albicans* and *C. utilise* with minimal inhibitory concentration of 39 and 78 μg.mL⁻¹, respectively. The antimicrobial potential of the oil was drastically reduced after it was subjected to the esterification reaction, indicating that the carboxyl group is responsible for the strong oil activity.

**Keyword:** Noni, *Candida*, *Morinda Citrifolia*, Fatty Acid, Antimicrobial.
INTRODUCTION

The fruit of the *Morinda citrifolia* L species (Rubiaceae) is popularly known as “noni,” a plant native to South-East Asia and Australia and cultivated in Polynesia, India, Americas. It is one of the fruits most used around the world for therapeutic purposes (Dixon et al., 1999).

In many countries, the *M. citrifolia* is used as food supplement. In folk medicine, it is used for treating diverse disorders such as cancer, diabetes, liver diseases, malaria, hypertension, tuberculosis, infection, arthritis and cardiovascular diseases (Ali et al., 2016). Previous studies have shown that the biological activity of *M. citrifolia* fruit is broad-spectrum including antimicrobial, anti-tumor, analgesic, antioxidant, anti-inflammatory, anxiolytic, antiarthritic, and antidiabetic activities (Assi et al., 2017; Krishnaiah et al., 2012). In in vivo tests, the ethanolic extract from the leaf and fruit from *M. citrifolia*, rich in phenolic compounds, showed hypotensive activity (Wigati et al., 2017). The aqueous fruit extract of *M. citrifolia* containing the gallic, caffeic, chlorogenic, and ellagic acids, showed antitumoral activity in Sarcoma 180 cells at a concentration of at 120 μg.mL⁻¹ (De Moraes et al., 2016). The compounds 2-caffeoyl-3-ketohexulofuranosonic acid γ-lactone and flavonoid glycoside isolated from *M. citrifolia* fruit juice are responsible for the anti-inflammatory activity from fruit juice (Youn et al., 2016).

*M. citrifolia* fruit extracts have been shown to have antimicrobial activity against virus, fungi and bacteria. Two new anthraquinones isolated from *M. citrifolia* ethanolic extract have shown anti-H1N1 activity with IC₅₀ values of 66.1 and 10.5 mM, respectively (Wang et al., 2016). Tissue extracts from *M. citrifolia* have shown antibacterial activity against *Escherichia coli* and the *Pseudomonas* ssp (Sunder et al., 2011). The hydro-alcohol leaf extract of *M. citrifolia* has exhibited activity against bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* with values of Inhibition zone diameter of 12, 11 and 7 mm, respectively (Nayak et al., 2015). *M. citrifolia* fruit extract at a concentration of 1000 μg.mL⁻¹ has demonstrated strong antifungal activity against *Candida albicans* with an Inhibition zone diameter of 16.6 mm, compared to the positive control – amphotericin B, which had a diameter of 20.6 mm (Barani et al., 2014).

The broad biological activity of the *M. citrifolia* specie may be associated with the chemical diversity of compounds isolated from its plant tissues, mainly from fruits. Some examples are: anthraquinones which are known for their biological potential, flavonoids, coumarins, steroids, lignans, alkaloids, fatty acids and triterpenes, totaling about 160 compounds identified (Krishnaiah et al., 2012; Potterat and Hamburger, 2007). Previous chemical studies have shown that *M. citrifolia* essential oils are rich in short-chain fatty acids, especially in hexanoic, octanoic and decanoic acids (Farine et al., 1996; Sang et al., 2002). There are only a few reports on biological activity of essential oils obtained from the *M. citrifolia* fruit including its antimicrobial activity. To our knowledge, there is only one study exploring the
antimicrobial potential of the oil which used only a preliminary test: the disc diffusion method (Brophy et al., 2008).

Here we report for the first time on the minimal inhibitory concentration (MIC) for M. citrifolia fruit essential oil for fungi, gram-positive bacteria and gram-negative bacteria, given that the oil showed strong antifungal activity against Candida albicans and C. utilis.

■ MATERIALS AND METHODS

Botanical Material

Ripe M. citrifolia fruits were collected in the city of Recife, Pernambuco state, northeastern Brazil, in August 2016 (Coordinates 8º00'54.5"S; 34º57'05.2"W). Botanical identification was carried out in the Sérgio Tavares Herbarium of the Pernambuco Federal Rural University and a voucher specimen was deposited there (53466).

Obtaining of Essential Oils

The essential oil from ripe M. citrifolia fruits (500 g) was obtained via hydrodistillation using a Clevenger-type apparatus. The distillation was carried out for 3 h and the distillate collected was treated with Na₂SO₄ to obtain the oil.

Chemical Analysis of Essential Oil

The essential oil from ripe M. citrifolia fruits (2 mg/mL) was analyzed by GC/MS with a programmed temperature of 60-240°C at 3°C min rate in a Gas Chromatograph (Perkin-Elmer Turbomass) equipped with a fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm) coated with DB-5 and coupled to a Mass Spectrometer (Varian 220-MS). The detailed conditions of GC/MS analysis have been previously reported by our research group (Rocha et al., 2016; Silva et al., 2016). Identification of the chemical constituents was carried out using the Van den Dool and Kratz (1963) equation and comparing with published mass spectra data (Adams, 2007).

Esterification Reaction of Essential Oil

A 350-mg aliquot of the essential oil obtained from ripe M. citrifolia fruit was dissolved in 30 ml of methanol and 1 ml of sulfuric acid was added. The reaction mixture was refluxed for 2 h, cooled, and then 20 ml of saturated sodium chloride solution (NaCl) was added. The mixture was fractioned three times with 25 mL of hexane and the hexane fraction was treated with anhydrous Na₂SO₄, then filtered and evaporated in a rotary evaporator to yield
315 mg (90%) of a dark brown oil. This oil was analyzed by GC/MS for identification of esterified oil compounds.

In Vitro Assay for Antimicrobial Activity

The antimicrobial potential of essential oil obtained from ripe *M. citrifolia* fruits was evaluated against the bacteria *Escheria coli* (UFPEDA 224), *Pseudomonas aeruginosa* (UFPEDA 416), *Bacillus subtilis* (UFPEDA 86) and *Staphylococcus aureus* (UFPEDA 02) as well as against the fungi *Candida albicans* (UFPEDA 1007) and *Candida utilis* (UFPEDA 1009). The microorganisms were maintained in nutrient agar (NA), and stored at 4°C. The antimicrobial activity evaluation was performed by determination of the values of minimum inhibitory concentrations (MIC) as previously reported (Rocha et al., 2016; Silva et al., 2016). The antimicrobials Metronidazol (10 μg.mL⁻¹) and Fluconazol (50 μg.mL⁻¹) were used as the positive control.

RESULT AND DISCUSSION

The essential oil of the fresh ripe *M. citrifolia* fruits was obtained by hydro-distillation for a yield of 0.14%. The chemical analysis of the essential oil by GC/MS identified nine compounds that corresponded to 88.6% of the total compounds detected (Table 1). The chemical composition of the oil has a high content of short-chain fatty acids, with octanoic and hexanoic acids being the major constituents with 38.7% and 20.0%, respectively.

The essential oil was submitted to an esterification reaction and methyl hexanoate (12.15%), methyl octanoate (83.38%) and methyl decanoate (4.46%) were obtained, confirming the presence of their carboxylic acids in the non-esterified essential oil. The octanoic and hexanoic acids have previously been identified in the essential oil from *M. citrifolia* fruit in a relative percentage of 60.2% and 16.3%, respectively (Brophy et al., 2008).

Considering the widespread use of noni fruit in popular medicine for various diseases, including those caused by microorganisms, the essential oil of the fruit was evaluated as antimicrobial against fungi and bacteria associated with human pathologies. The values of MICs essential oil from fresh ripe *M. citrifolia* fruits against bacteria and fungi are shown in Table 2. This oil showed inhibitory activity for all microorganisms tested with MICs values ranging from 39 μg.mL⁻¹ to 625 μg.mL⁻¹, with the lowest value for the fungus *C. albicans* and the highest value for gram-negative bacteria *P. aeruginosa*, respectively. According to criteria in the literature (Champagnat et al., 2006), samples from natural products showing a MIC lower than 100, ranging from 100–500, 500–1000 and over 1000 μg.mL⁻¹ are characterized as potent, mod- erated, weak, or not active, respectively.
Table 1. Chemical constituents identified in the *M. citrifolia* fruit essential oil.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Al</th>
<th>Ar</th>
<th>Relative amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl (3E)-hexenoate</td>
<td>916</td>
<td>927</td>
<td>6.1</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>980</td>
<td>967</td>
<td>20.0</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>993</td>
<td>997</td>
<td>0.2</td>
</tr>
<tr>
<td>Pentyl isobutanoate</td>
<td>1058</td>
<td>1049</td>
<td>1.5</td>
</tr>
<tr>
<td>Nonan-2-one</td>
<td>1086</td>
<td>1087</td>
<td>0.3</td>
</tr>
<tr>
<td>Methyl octanoate</td>
<td>1119</td>
<td>1123</td>
<td>17.2</td>
</tr>
<tr>
<td>Octanoic acid</td>
<td>1171</td>
<td>1167</td>
<td>38.7</td>
</tr>
<tr>
<td>Decanoic acid</td>
<td>1350</td>
<td>1364</td>
<td>1.0</td>
</tr>
<tr>
<td>Isoamyl octanoate</td>
<td>1446</td>
<td>1442</td>
<td>0.7</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>85.7%</td>
</tr>
</tbody>
</table>

Compounds are listed in ascending order of their retention times. Al: Arithmetic index. Ar: Arithmetic index as determined on nonpolar DB-5 column. Arithmetic index of the literature.

Table 2. Minimum inhibitory concentration values of *M. citrifolia* fruit essential oil for bacteria and fungi.

<table>
<thead>
<tr>
<th>CMIC (µg.mL⁻¹)</th>
<th>Bacteria</th>
<th>Oil</th>
<th>Esterified oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
<td>312.5</td>
<td>&gt;2500</td>
</tr>
<tr>
<td></td>
<td><em>B. subtilis</em></td>
<td>156.2</td>
<td>&gt;2500</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td>312.5</td>
<td>2500</td>
</tr>
<tr>
<td></td>
<td><em>P. aeruginosa</em></td>
<td>625</td>
<td>2500</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. albicans</em></td>
<td>39.0</td>
<td>1250</td>
</tr>
<tr>
<td></td>
<td><em>C. utilis</em></td>
<td>78.1</td>
<td>1250</td>
</tr>
</tbody>
</table>

Table 2. also shows the MICs values for the esterified essential oil. The antimicrobial potential of the oil is drastically reduced after the esterification reaction, indicating that the short-chain fatty acids identified are responsible for the strong oil activity.

Carboxyl groups are present in the chemical structures of the main commercially available antibiotics including those of natural and synthetic origin such as penicillin and ciprofloxacin.

A previous study with several fatty acids showed that decanolic and dodecanoic acids act as fungicide against *C. albicans* and can be used for the treatment of infections caused by this pathogen. The analysis by transmission electron microscopy on *C. albicans* showed the cytoplasm disorganized and shrunken because of a disrupted or disintegrated plasma membrane, a similar mechanism of action was also observed for (Z)-9-heptadecenoic acid, which inhibits the growth and germination of the fungi *Phytophthora infestans* and *Idriella bolleyi* (Bergsson et al., 2001; Carballeira, 2008).

**CONCLUSION**

These MICs values revealed that essential oil of the noni fruit is a potent natural antifungal and can be considered a promising source for the alternative treatment of fungal diseases.
ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

LEGH was responsible for the collection of fruit sample, oil obtaining and the phytochemical studies. GBB realized the antimicrobial activity. CSR designed the study and supervised the laboratory work.

REFERENCES


