SHORT COMMUNICATION

In vitro screening by phototoxic properties of Eugenia uniflora L., Momordica charantia L., Mentha arvensis L. and Turnera ulmifolia L.

Henrique Douglas Coutinho1*, José Galberto Martins Costa2, José Pinto Siqueira Jr3 and Edeltrudes Oliveira Lima4

Available online at http://www.ufrgs.br/seerbio/ojs/index.php/rbb/article/view/1421

Received: November 03 2009    Received after revision: January 26 2010    Accepted: January 26 2010

ABSTRACT: (In vitro screening by phototoxic properties of Eugenia uniflora L., Momordica Charantia L., Mentha arvensis L. and Turnera ulmifolia L.). Ethanol extracts of Mentha arvensis L., Momordica charantia L., Turnera ulmifolia L. and Eugenia uniflora L. were screened for light – activated antibacterial activity against strains of Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Triplicate assays were conducted with and without exposure to UV-A radiation to test for light-activated or light-enhanced activity. Only the ethanol extract of E. uniflora and M. charantia showed antibacterial activity against an E. coli strain when exposed to UV-A light. The results represent a first report of the light-mediated antimicrobial activity of E. uniflora and M. charantia and suggest that phytochemical investigations may be warranted.

Key words: Momordica charantia, Eugenia uniflora, light-mediated antibacterial activity, UV-A, UV light.

INTRODUCTION

Medicinal plants have been the subject of intense research due to their potential as sources of commercial drugs or as lead compounds in drug development. There is a growing interest in the influence of biologically active compounds isolated from plants in the treatment of diseases caused by microorganisms (Austin et al. 1999). New aspects of the photochemistry and photobiology of natural products, including their potential as therapeutic agents, have been reviewed (Lopez et al. 2001). An increasing number of natural products from plants have been shown to exhibit light-mediated biological activity against viruses, microorganisms, cells and insects (Towers et al. 1997). Although there is a great amount of published data regarding the antimicrobial properties of medicinal plants, there is no information on light-activated biological activities from this natural resource (Tip-pyang et al. 2000). Focusing on the concept of photochemistry and photochemotherapy, we examined here two potentially useful Brazilian medicinal plants and found their extracts to show light-mediated antimicrobial activities.

Eugenia uniflora L. (Myrtaceae) fruits and leaves are used as foods and in folk medicine, respectively, because of their traditionally purported antimicrobial activity (Souza et al. 2004), and have been extensively studied because of their biological activities (Sharma et al. 2006). E. uniflora has yielded compounds such as the flavonoids myricitrin, quercetin and its 3-L-rhamnoside quercitrin, steroids and/or triterpenoids, mono and triterpenic compounds, tannins, anthraquinones and phenols, cineole and essential oils (Wazlawik et al. 1997). Momordica charantia L. (Cucurbitaceae) is a climber known as bitter melon, which grows worldwide. Several flavonoids with pharmacological and biological activities have been identified in M. charantia (Grover & Yadav 2004, Coutinho et al. 2009a, b).

The present investigation was a preliminary screening for phototoxic activity in natural products from plants used as food and as medicines in Brazil. In this study, we tested extracts of Mentha arvensis L. (Labiatae), Momordica charantia L. (Cucurbitaceae), Turnera ulmifolia L. (Turneraceae) and Eugenia uniflora L. (Myrtaceae) as a source of phototoxic compounds against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa.

1. Laboratório de Pesquisas em Produtos Naturais, Departamento de Ciências Biológicas, Centro de Ciências Biológicas e da Saúde, Universidade Regional do Cariri. CEP 63105-000, Crato, CE, Brazil.
2. Faculdade Leão Sampaio, Juazeiro do Norte, CE, Brazil.
3. Laboratório de Genética de Microorganismos, Universidade Federal da Paraíba. João Pessoa (PB), Brazil.
4. Laboratório de Micologia, Universidade Federal da Paraíba. João Pessoa, PB, Brazil.

* Author for correspondence. E-mail: hdmcoutinho@gmail.com
MATERIALS AND METHODS

Strains

The bacterial strains used were: Escherichia coli (ATCC 10536 and 8539), Pseudomonas aeruginosa (ATCC 25619 and 9027) and Staphylococcus aureus (ATCC 25923 and 6538). All strains were maintained on heart infusion agar slants (HIA, Difco Laboratories Ltd.) and prior to assay, the cells were grown overnight at 37ºC in brain heart infusion (BHI, Difco Laboratories Ltd.).

Plant material

Leaves of Mentha arvensis L., Momordica charantia L., Turnera ulmifolia L. and Eugenia uniflora L. were collected in the county of Crato, Ceará State, Brazil. The plant material was identified and a voucher specimen was deposited with the respective numbers at the Herbarium “Dârданo de Andrade Lima” of Universidade Federal do Cariri – UNICAR (Table 1). Aerial parts (200 g) were dried at room temperature and powdered. The material was extracted by maceration using 1 L of 95% ethanol as solvent at room temperature, and the homogenate was allowed to stand for 72 h at room temperature. The extracts were then filtered and concentrated under vacuum in a rotary evaporator (model Q-344B – Quimis, Brazil) and ultrathermal bath (model Q-214M2 - Quimis, Brazil). Each 200 g of aerial parts yielded 5-6 g of extract. The dry extract material was dissolved in DMSO for the analysis.

Phototoxic assay

Assays were performed according to Coutinho et al. (2009c). As positive controls, a disk of norfloxacin was used as a standard antibiotic for bacteria with phototoxic properties. 8-Methoxypsoralen (8-MOP – 10 mg/mL) dissolved in water was utilized as a positive control requiring light for activation. Twenty microliters of each extract were added to blank disks. These disks were placed on the surface of the medium inoculated with bacteria by the spread plate method. To monitor for light-activated antimicrobial activities, two replicate experiments were carried out. One replicate plate was exposed to ultraviolet (UV) light (5 W/m²; 320-400 nm from four Sylvania F20T12-BLB lamps, maximum emission at 350 nm) for 2 h, while the other was kept in the dark. The plates were incubated at 37ºC overnight; the inhibition zones were determined using a pachymeter and recorded as shown in Table 2.

RESULTS AND DISCUSSION

The extracts of M. arvensis and T. ulmifolia do not show phototoxic activity against the bacterial strains assayed. The exposure to UV-A light had a considerable effect on the activities of M. charantia and E. uniflora extracts against the E. coli strain ATCC8539. Neither extract was phototoxic against S. aureus and P. aeruginosa strains (Table 2). As far as we know, no phototoxic effect has been reported for natural products of M. charantia and E. uniflora.

Several substances exhibit phototoxicity when exposed to visible or UV light, being referred as photosensitizers. These compounds are found in various families of plants and fungi, likely as defense agents against natural enemies or parasites (Towers et al. 1997, Cheeptham & Towers 2002, Kang et al. 2007). The phototoxic activity is due the production of free radical or by affecting cells directly (Foote 1991). Some compounds with phototoxic activity have already been shown to possess biological activity when photoaivated to be used, as hypericin and hypocresin, that showed anti-HIV and antitumor activities (Towers et al. 1997), while several other natural products from plants demonstrated antibacterial activity with light exposure (Cheeptham & Towers 2002, Kang et al. 2007, Coutinho et al. 2009c).

The results of the present study represent the first report of light-mediated antimicrobial activities of phytochemicals from the Families Cucurbitaceae and Myrtaceae, as well as in the genus Eugenia and Momordica, suggesting that E. uniflora and M. charantia could be used as a source of natural products with phototoxic activities, which could be an interesting alternative either in the treatment of skin diseases, such as psoriasis and vitiligo, or to the use of 8-MOP in the PUVA (Psoralen + UVA) treatment.

ACKNOWLEDGEMENT

This work was supported by the agencies CNPQ and FAPESQ/PB.

Table 1. Botanical families, species and voucher number of the plants used in this study.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turneraeae</td>
<td>Turnera ulmifolia</td>
<td>#1618</td>
</tr>
<tr>
<td>Lamiaeae</td>
<td>Mentha arvensis</td>
<td>#2886</td>
</tr>
<tr>
<td>Cucurbitaeae</td>
<td>Momordica charantia</td>
<td>#703</td>
</tr>
<tr>
<td>Myrtaceae</td>
<td>Eugenia uniflora</td>
<td>#3106</td>
</tr>
</tbody>
</table>

Table 2. Light-mediated antimicrobial activity of ethanol extracts of species.

<table>
<thead>
<tr>
<th></th>
<th>SA6538</th>
<th>SA25923</th>
<th>EC8539</th>
<th>ECI0536</th>
<th>PA25619</th>
<th>PA9027</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV-</td>
<td>UV+</td>
<td>UV-</td>
<td>UV+</td>
<td>UV-</td>
<td>UV+</td>
</tr>
<tr>
<td>EEMA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EEEU</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>EEMC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>EETU</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NOR*</td>
<td>32</td>
<td>35.5</td>
<td>27.5</td>
<td>30.5</td>
<td>24</td>
<td>30.5</td>
</tr>
<tr>
<td>8MOPb</td>
<td>-</td>
<td>18</td>
<td>-</td>
<td>13</td>
<td>-</td>
<td>14.5</td>
</tr>
</tbody>
</table>

EEMA, Ethanol extract of Mentha arvensis; EEEU, Ethanol extract of Eugenia uniflora; EEMC, Ethanol extract of Momordica charantia; EETU, Ethanol extract of Turnera ulmifolia; UV-, without UV irradiation; UV+, with UV irradiation; a. Norfloxacin (10 mg/disk), positive standard; b. 8-Methoxyl-8psoralen (10 mg/mL); - No inhibition zones.
REFERENCES


