



SHORT COMMUNICATION

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

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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.

RESUMO: (Screening *In vitro* por propriedades fototóxicas de *Eugenia uniflora* L., *Momordica charantia* L., *Mentha arvensis* L. e *Turnera ulmifolia* L.). Extratos etanólicos de *Mentha arvensis* L., *Momordica Charantia* L., *Turnera ulmifolia* L. e *Eugenia uniflora* L. foram avaliados quanto a sua atividade antibacteriana ativada pela luz contra linhagens de *Staphylococcus aureus*, *Escherichia coli* e *Pseudomonas aeruginosa*. Experimentos em triplicata foram conduzidos com e sem exposição à radiação UV-A para verificar a presença de atividade antibacteriana ativada ou aumentada pela luz. Apenas os extratos etanólicos de *E. uniflora* e *M. charantia* apresentaram atividade contra uma linhagem de *E. coli* quando exposta à luz UV-A. Os resultados representam o primeiro relato de atividade antimicrobiana mediada pela luz de *E. uniflora* e *M. charantia*.

Palavras-chave: *Momordica charantia*, *Eugenia uniflora*, atividade antibacteriana mediada pela luz, UV-A, luz UV.

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 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-ramnoside quercitrin, steroids and/or triterpenoids, mono and triterpenic compounds, tannins, anthraquinones and phenols, cineol 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*.

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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 37°C 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 "Dárdano de Andrade Lima" of Universidade Regional do Cariri – URCA (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 photoactivated 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 37°C overnight; the inhibition zones were determined using a pachymeter and recorded as shown in Table 2.

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

Family	Species	Number
Turneraceae	<i>Turnera ulmifolia</i>	#1618
Lamiaceae	<i>Mentha arvensis</i>	#2886
Cucurbitaceae	<i>Momordica charantia</i>	#703
Myrtaceae	<i>Eugenia uniflora</i>	#3106

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 phototoxins or 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 photoactivated to be used, as hypericin and hypocrellin, 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 2. Light-mediated antimicrobial activity of ethanol extracts of species.

	SA6538		SA25923		EC8539		EC10536		PA25619		PA9027	
	UV-	UV+	UV-	UV+	UV-	UV+	UV-	UV+	UV-	UV+	UV-	UV+
EEMA	-	-	-	-	-	-	-	-	-	-	-	-
EEEU	-	-	-	-	-	12	-	-	-	-	-	-
EEMC	-	-	-	-	-	10	-	-	-	-	-	-
EETU	-	-	-	-	-	-	-	-	-	-	-	-
NOR ^a	32	33.5	27.5	30.5	24	30.5	28	29.5	25	27.5	24.5	30
8MOP ^b	-	18	-	13	-	14.5	-	13	-	11	-	10.5

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

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