Revista Brasileira de Farmacognosia (2021) 31:121–125
https://doi.org/10.1007/s43450-021-00129-6

SHORT COMMUNICATION

Anti-Zika Activity of *Ouratea semiserrata* and Dereplication of Its Constituents

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Received: 24 August 2020 / Accepted: 14 January 2021 / Published online: 5 February 2021

Abstract

Zika virus is an arbovirus that has vector mosquitoes of the genus *Aedes*. In adult humans, the infection may be asymptomatic or present mild symptoms such as itching and low fever. However, the infection is associated with other severe problems, which encouraged investigations for an effective treatment against this virus. This work evaluated the potential anti-Zika virus effect of the ethanolic extract of *Ouratea semiserrata* (Mart. & Nees) Engl., Ochnaceae, a medicinal plant popularly used in Brazil for the treatment of viral infections. The extract of the stems was prepared by cold percolation using ethanol as solvent and its content dereplicated by ultra-high-performance liquid chromatography-diode array detector-tandem mass spectrometry. Phenolic compounds including rutin, catechin, and epicatechin were identified as the major constituents. The antiviral activity was tested *in vitro* against Zika virus by the MTT colorimetric method. The ethanol extract inhibited the viral replication cycle with an EC_{50} of 37.5 μg/ml, and at the concentration of 100 μg/ml, a 100% inhibition of the viral cytopathic effect was obtained. Rutin and epicatechin inhibited viral cytopathic effect in Vero cells with $EC_{50} > 50.00$ μg/ml.

Keywords Anti-arboviral activity · Proanthocyanidins · Ochnaceae · Natural products · *Flavivirus* · Flaviviridae

Introduction

Arboviruses have been a major public health problem in the world. Since viruses belonging to the Flaviviridae family play an important role in the human diseases associated with this type of virus which include dengue viruses, yellow fever, and Nile fever. Zika virus (ZIKV), a type of arbovirus from the genus *Flavivirus*, is a single-strand RNA virus with two known strains, one from Africa and another from Asia (Haddow et al. 2012). The virus is transmissible by *Aedes aegypti* and *Aedes albopictus* mosquito bites; these mosquitoes are the same vectors for dengue and yellow fevers (Prophiro et al. 2011). The main symptoms are similar to dengue fever, which are muscle pain, fever, eye pain, prostration, and maculopapular rash. The fever is usually low, and in most of the cases, the infection is mild and asymptomatic. The infection is strongly associated with neurological disorders such as Guillain-Barré syndrome and also with microcephaly in newborns of pregnant women infected with ZIKV (Barrows et al. 2016; Fauci and Morens 2016). The most accurate diagnosis method to detect the ZIKV infection is by RT-PCR, but this technique can generate a considerable amount of false negative results (Luz et al. 2015). The Pan American Health Organization registered 754,460 suspected and confirmed cases of Zika virus infection between January 2015 and March 2017 in the Americas. Brazil represents 46% of cases and the South American continent 70% of cases in the Americas (Hills et al. 2017). At the moment, there is no available drug to treat patients infected by ZIKV and the treatment is just for the symptom amelioration (Barrows et al. 2016). Therefore, the search for new drugs is urgent in this scenario.

In the context of research on natural products obtained from plants, members of *Ouratea* genus, Ochnaceae, are considered as a good source of flavonoids (Fidelis et al. 2014). It

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has been reported that extracts of *Ouratea castaneifolia* (DC.) Engl. and *O. semiserrata* (Mart. & Nees) Engl. demonstrated activities against *Vaccinia virus* and encephalomyocarditis viruses (Brandão et al. 2011). In the present work, the presence of flavonoids and proanthocyanidins was identified in the ethanolic extract from the stem of *O. semiserrata* by liquid chromatography coupled to diode array detection and electrospray ionization tandem mass spectrometry. The cytotoxicity and antiviral activity against ZIKV of this extract was evaluated in Vero cells line by MTT colorimetric assay.

Materials and Methods

Ouratea semiserrata (Mart. & Nees) Engl., Ochnaceae, was collected in Belo Horizonte, Minas Gerais, Brazil (19° 54′ 46.7928″ S and 43° 56′ 27.3588″ W). The plant was taxonomically identified by Dr. J. A. Lombardi, Department of Botany, Institute of Biosciences, UNESP, Rio Claro, Brazil. A voucher specimen was deposited at the BHCB/UFMG, Belo Horizonte, Minas Gerais, Brazil, under the number accession 42166. After drying in an air-circulating oven at 40 °C for 72 h, the plant material, 836 g of stems, was ground and extracted by percolation with 96% EtOH at room temperature with 16% yield. Rutin, catechin, and epicatechin were purchased from the Sigma-Aldrich company. The LC-DAD-MS and LC-ESI-MS/MS analyses were performed as described by Reis et al. (2020).

Vero cells (ATCC CCL-81) were cultured in Dulbecco's modified Eagle's medium high glucose (DMEM-HG, Cultilab, Campinas, SP, Brazil) at 37 °C, in 5% CO_2 atmosphere, supplemented with 5% fetal bovine serum, 50 μg/ml gentamicin, and 100 U/ml penicillin, and 5 μg/ml of amphotericin B (Brandão et al. 2013). ZIKV (PE-243/215 Asian strain) was kindly donated by Dr. E. Kroon (UFMG, Belo Horizonte, Brazil). The cytotoxicity test was performed by the MTT colorimetric assay to obtain CC_{50} values; the concentration of the compound exhibits 50% cytotoxicity. Vero cells (2×10^4 cells per well) were exposed to different concentrations (400 to 0.4 μg/ml) of ethanolic extract of stem and compounds identified in this species for 72 h (Brandão et al. 2013). Ribavirin was used as positive control with EC_{50} = 94.47 ± 2.70 μg/ml (Reis et al. 2020). After incubation, cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Merck) assay at a concentration of 2 mg/ml in PBS (Brandão et al. 2013). The CC_{50} values of ethanol extract from *O. semiserrata* stems, catechin, epicatechin, and rutin were used as a parameter for the antiviral screening, using a non-cytotoxic concentration to perform the tests in Vero cells infected by ZIKV.

The antiviral activity measured by the median effective dose (EC_{50}) of compounds and ethanolic extract of *O. semiserrata stem* was evaluated by the MTT assay. The cell monolayer (2.0×10^4 cells per well) was infected with ZIKV suspensions (MOI = 1.1). Dilutions of the compounds and ethanolic extract in non-cytotoxic concentrations were added to the wells after viral infection. The plates were incubated at 37 °C in humidified 5% CO_2 atmosphere for a period of 72 h. The 50% inhibitory concentration of the viral effect (EC_{50}) for compounds and ethanolic extract were calculated from concentration-effect-curves after nonlinear regression analysis (Brandão et al. 2013). In order to confirm the anti-ZIKV effect of *O. semiserrata* extract and the compounds, a six-well plate assay using the same parameters as those used in the 96-well plate experiments was performed and photographs were taken under an optical microscope. In the present article, the following were arbitrarily established: very active (EC_{50} < 50 μg/ml), moderately active (50 < EC_{50} < 100 μg/ ml), low activity (EC_{50} > 100 μg/ml).

Statistical calculations were carried out with the GraphPad prism 5.0 software package (Statistica). Results are expressed as the mean ± S.E.M. of 4 independent experiments. Student's *t* test was used for statistical analyses; *p* values > 0.05 were considered to be significant.

Results and Discussion

Dereplication analyzes were performed by UPLC-DAD-MS allowing partial identification of ten known compounds and later some were confirmed with coelution with of authentic samples. Analysis of first-order MS spectra recorded for each peak together with MS^2 experiments in positive and negative ESI mode, UV comparison, and retention time led to the following structure assignments (Figure S1 and Table S1). For positive identification and characterization of flavan-3-ol monomers, dimmers, trimers, and tetramers, the following points were considered: UV absorption close to 280 nm (λ_{max}), and molecular ion peaks in positive and negative ion ESI mode of MS. The fragmentation pathway heterocyclic ring fission (HRF) and retro-Diels–Alder (RDA) fragmentation give information about the hydroxylation of the B-rings and bonds between two monomeric units, and quinone methide (QM) fragmentation defines the two monomeric units and especially the base unit (Jaiswal et al. 2012; Abad-García et al. 2009). Experimental data and comparison with literature data suggested that compound with Rt 2.17 min corresponded to an (*epi*)catechin monomer and compounds with Rt 1.94 and 2.68 min were assigned as dimers B-type linkages of (*epi*)-catechin-(*epi*)catechin, respectively (Jaiswal et al. 2012). Compounds with Rt 1.91, 2.19, and 2.32 min were assigned as trimers B-type linkages of (*epi*)catechin-(*epi*)-catechin-(*epi*)catechin, respectively. Compound with Rt 1.85 min is assigned as a proanthocyanidin tetramer [(*epi*)-catechin-(4,8)-(*epi*)catechinn-(4,8)-(*epi*)catechin-(4,8)-(*epi*)-catechin]. For the identification of flavonoids, the data

obtained experimentally for these compounds were also compared with the data described in the literature. The three identified flavonoids have been previously described in species of the genus *Ouratea* (Suzart et al. 2012; Velandia et al. 2002): vitexin with Rt 2.62 min, lanaraflavone with 4.76, and rutin with 2.41 min. Chemical structures for all dereplicated compounds are included as supplementary material.

In the cytotoxicity assays, eight different concentrations ranging from 400 to 3.1 μg/ml for *O. semiserrata* and 50 to 0.4 μg/ml for catechin, epicatechin, and rutin were used. The CC_{50} results were determined from the values obtained in distinct experiments by nonlinear regression using GraphPad prism 5.0 software package (Statistical). After the antiviral assay with ZIKV in a 96-well plate, *O. semiserrata* exhibited $EC_{50} > 37.5$ μg/ml with 1.30 selectivity index showing itself to be active against infection by ZIKV. Epicatechin presented EC_{50} 140.70 ± 1.61 μg/ml with selectivity index > 3.55 showing low antiviral activity against infection by ZIKV. Catechin and rutin both presented $EC_{50} > 50.00$ μg/ml with selectivity index > 10.00 and > 4.00, respectively, showing moderate activity against infection by ZIKV. In relation to the six-well plate assay shown in Fig. 1, at 100.0 μg/ml of ethanolic extract of *Ouratea semiserrata*, a 100% protection of the cell monolayer was observed, demonstrating once again the anti-ZIKV activity of this species. At 50 μg/ml, rutin and catechin showed protection of 64.6 and 59.2%, respectively.

The chemical profile of the species *Ouratea semiserrata* analyzed by UPLC-DAD-ESI/MS as shown in Table S1 corroborates what is found in the scientific literature where it highlights flavonoids as chemical markers of the genus *Ouratea* (do Nascimento et al. 2009). Three flavonoids were detected: one flavone (vitexin), one biflavonoid (lanaraflavone), and one flavonol (rutin). A total of four proanthocyanidin were also detected. Previous studies showed the presence of flavones in extracts of species of the Ochnaceae family, mainly in species of the genus *Ouratea* (Fidelis et al. 2014). Vitexin was identified in the methanolic extract of *Ouratea hexasperma* flowers (Suzart et al. 2012). There are studies showing *in vitro* antiviral activity of vitexin against parainfluenza 3 virus (Li et al. 2002). Through this data, it is suggested that the presence of vitexin in the ethanolic extract of *Ouratea semiserrata* may be linked to the anti-Zika virus effect presented in this research. Lanaraflavone and other chemically similar compounds were identified in the methanolic extract of *Ouratea hexasperma* leaves and methanolic extract of *Ouratea semiserrata* branches (Velandia et al. 2002) but there is no report of antiviral activity for this molecule.

Flavonol rutin was detected in the species *Ouratea semiserrata* (Velandia et al. 2002) which corroborates the finding in this article for the same plant species. This molecule is already well studied as a natural product and several pharmacological activities have been demonstrated. Regarding the antiviral activities attributed to rutin described in the literature, we can highlight the activity against the H5N1 avian influenza strain in MDCK cells (Ibrahim et al. 2013) and a study conducted by Lim et al. (2017) demonstrated the ability of rutin to inhibit the NS2B-NS3 protease, essential for the replication of the Zika virus, which may suggest that anti-Zika virus effect presented in this work may be related to the inhibition of these proteases. The study also demonstrated this same effect on

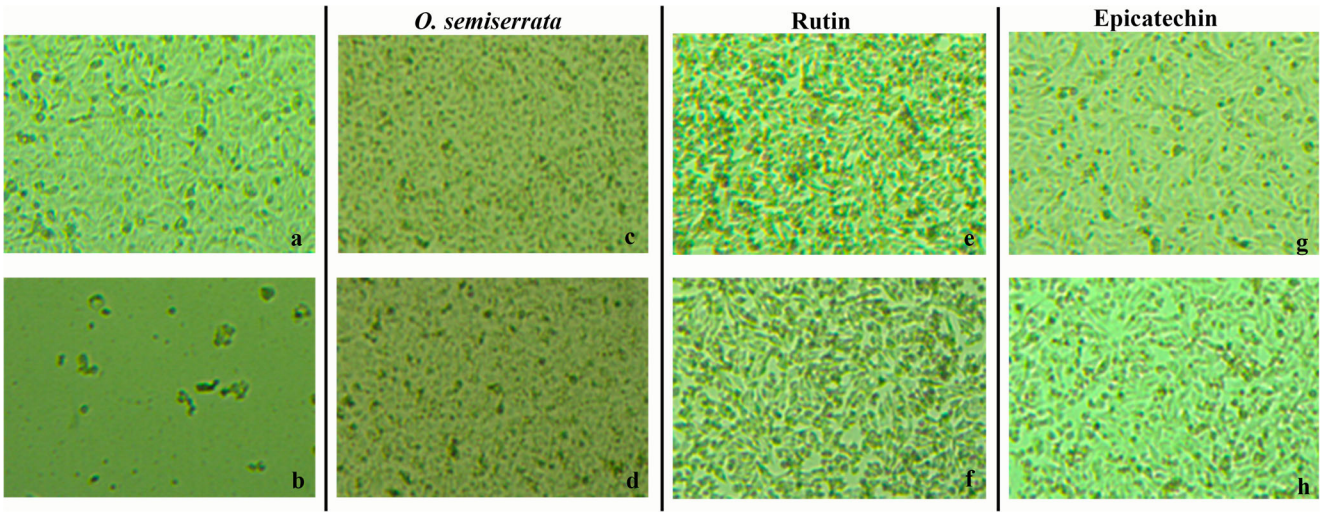

Fig. 1 Antiviral effect against ZIKV in Vero cells treated of ethanolic extract of *Ouratea semiserrata*, rutin, and epicatechin. Subtitle: Vero cells were infected with ZIKV, treated with *O. semiserrata* leaves ethanolic extracts and photographed after 72 h of infection. **a** Uninfected and untreated cells. **b** Infected and untreated cells. **c** Cells uninfected and treated with *O. semiserrata* extract (100 μg/ml). **d** Cells infected and treated with *O. semiserrata* extract (100 μg/ml). **e** Cells uninfected and treated with rutin (50 μg/ml). **f** Cells infected and treated with rutin (50 μg/ml). **g** Cells uninfected and treated with epicatechin (50 μg/ml). **h** Cells infected and treated with epicatechin (50 μg/ml), magnification, × 100

epicatechin gallate. The presence of proanthocyanidins was already described in the species *Ouratea semiserrata* (Côrtes et al. 2002). Some of the proanthocyanidins detected in this work present antiviral activity reported for some viruses such as dengue virus (DENV) (Trujillo-Correa et al. 2019), which may be linked to the antiviral effect registered in *O. Semiserrata* extract.

Conclusions

The results reveal the presence of proanthocyanidins and flavonoids in *Ouratea semiserrata*. Our findings are the first report on the chemical and antiviral activity of *O. semiserrata* constituents. Our results are in line with the traditional use of *Ouratea* species as anti-infectious agents in different South American countries and might be of interest for the development of standardized antiviral phytomedicines.

Acknowledgments Prof. A.B. Oliveira and F.C. Braga would like to express their gratitude to the Laboratório de Fitoquímica da Faculdade de Farmácia, UFMG for access to the equipment for chromatographic analysis. Thanks are also expressed to G.P. Skiminn for the authorization to reproduce the plant material image.

Authors' contributions GMF carried out the experiments and wrote the manuscript. GCB outlined and guided the project and revised and corrected the manuscript. GHBS supervised the chemical analysis. BMS collaborated with biological assays that involved cell culture. ABO facilitated and participated in the chromatographic analyses.

Funding This work was supported by funds from Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Brazil), process number CDS/APQ-01529-15; Conselho Nacional de Desenvolvimento Científico e Tecnológico (Brazil), INCT Dengue (Dengue IMH), process number N.: 465425/2014-3; Pró-Reitoria de Pesquisa e Pós-Graduação da UFOP, process number 23109.003517/2018-85 and Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (Brazil).

Compliance with ethical standards

Conflict of Interest The authors declare no conflict of interest.

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