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Short communication

Eichhornia crassipes: an advantageous source of shikimic acid

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ABSTRACT

The water hyacinth (*Eichhornia crassipes* (Mart.) Solms, Pontederiaceae) is considered as one of the most productive plants on earth, and an aquatic weed, which causes serious environmental problems. In this study, this species is presented as an alternative of a renewable source of shikimic acid. Although this acid is an important intermediate in the biosynthesis of aromatic compounds in plants and microorganisms, its occurrence is described for the first time in a species of the Pontederiaceae family. Shikimic acid is the lead compound for the production of the antiviral agent oseltamivir phosphate (Tamiflu®). Semi-quantitative analyses of the plant extracts by HPLC-PDA showed that the aerial parts of *E. crassipes* contain higher shikimic acid concentration (0.03%-2.70% w/w) than the roots (0.05%-0.90% w/w), and that methanol is a better solvent than water for shikimic acid extraction.

Introduction

The water hyacinth, *Eichhornia crassipes* (Mart.) Solms, Pontederiaceae, is a free floating aquatic plant native of Brazil. This plant is known as one of the world's most invasive aquatic weeds due to its fast spread and congested growth, and a source of biomass (over 60 kg m^{-2}) (Malik, 2007). Although the water hyacinth is often seen as a weed, it may be used as a phytoremediation agent, because of its ability to grow in heavily polluted water and its capacity to accumulate metals, radionuclides and other pollutants (Malik, 2007; Rai, 2008; Mahamadi, 2011). Investigations on biofuel and compost production from *E. crassipes* have been described in the literature (Gunnarsson and Petersen, 2007; Ganguly et al., 2012). It has been reported that extracts of *E. crassipes* show antimicrobial (bacterial and fungal), anti-algal (green microalgae and cyanobacteria), and antioxidant activities (Malik, 2007).

Phytochemical studies on *E. crassipes* have led to the isolation and identification of more than thirty compounds, including sterols, flavonoids, and phenalenone-type compounds (Toki et al., 2004; Hölscher and Schneider, 2005; DellaGreca et al., 2009; Lalitha et al., 2012). In this paper we describe for the first time the presence of shikimic acid (**1**) in the Pontederiaceae family.

Shikimic acid ((3R, 4S, 5R)-3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid) (**1**) is a naturally occurring organic compound, from which its anionic form, shikimate, is an important intermediate in the biosyntheses of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) of plants and microorganisms (Herrmann and Weaver, 1999; Maeda and Dudareva, 2012). Recent reviews on natural, biotechnological, and synthetic sources, as well as pharmacological applications of shikimic acid, have been published (Avula et al., 2009; Bochkov et al., 2012; Estévez and Estévez, 2012; Ghosh et al., 2012; Rawat et al., 2013; Quiroz et al., 2014). This acid

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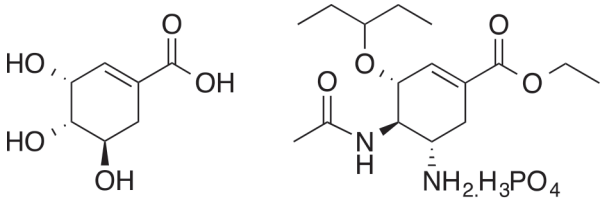
has interesting biological properties, displaying antioxidant, anticoagulant, antibacterial, anti-inflammatory, and analgesic activities (Estévez and Estévez, 2012; Rawat et al., 2013).

Shikimic acid (**1**) is a lead compound in the manufacture of the drug oseltamivir phosphate, commercially known as Tamiflu® (**2**), which is an oral antiviral used to treat influenza viruses, as A H5N1 (avian influenza) and A H1N1 (swine influenza). According to Roche Pharmaceutical Company, the major bottleneck in Tamiflu production is the availability of **1** (The Roche Group, 2014). Commercially, it is mainly obtained from the seeds of Chinese star anise (*Illicium verum*) and by fermentation process (genetically engineered *Escherichia coli* strains) (Enrich et al., 2008).



1 **2**

In order to contribute to the advances regarding the utilization of water hyacinth, and encouraging the householders to harvest the weed, keeping it under control, we focus on the shikimic acid metabolite. This report describes a semi-quantitative analysis of ten extraction conditions to yield **1** from *E. crassipes*, and compares the concentration of **1** from the extracts of roots and aerial parts of the plant.

Materials and methods

The NMR experiments (^{1}H and ^{13}C) were performed on a Varian INOVA 500 spectrometer (11.7 T) at 500 MHz (^{1}H) and 126 MHz (^{13}C), using deuterated solvents (D_2O and DMSO-d_6) (P 99.9% D). Reported δ are relative to TMS. IR spectrum was obtained on a Perkin-Elmer 1600 FT-IR spectrometer using a KBr disc. Optical rotation was measured on a Perkin Elmer 341 LC polarimeter. HPLC analyses were performed with a Jasco LC-Net II/ADC, equipped with photodiode array (MD-2018 Plus) detector, on a Varian Chrompack OSD column (250 x 4.6 mm, 5 µm).

Plant material

Eichhornia crassipes (Mart.) Solms, Pontederiaceae, was collected in Araraquara, SP, Brazil, and identified as *Eichhornia crassipes* (Mart.) Solms by Dr. Maria do Carmo E. Amaral. A voucher specimen (UEC 164949) was deposited at the herbarium of the Universidade Estadual de Campinas, Campinas, SP, Brazil.

Isolation and identification of shikimic acid (1, standard compound)

The whole plants were dried (1756 g), ground, and extracted at room temperature with ethanol. The solution was concentrated to dryness at reduced pressure yielding 95.9 g of the crude extract. A portion of this extract (85.8 g) was suspended in CH_3OH-H_2O (4:1) and partitioned with *n*-hexane, CH_2Cl_2, and EtOAc, successively. The portion soluble in EtOAc (2.23 g) was subjected to CC over Sephadex LH-20 and eluted with CH_3OH to give **1** (564.2 mg). The ^{1}H and ^{13}C NMR, UV, and IR data of **1** agree with those reported in the literature (Bochkov et al., 2012). The specific rotation of **1** ($[\alpha]_D$ –147 [*c* 0.42, CH_3OH]) also agrees with the literature ($[\alpha]_D$ –159 [*c* 0.7, CH_3OH, Wada et al., 1992]).

Shikimic acid calibration curve

The standard compound was analyzed by HPLC-PDA. Elution conditions: flow rate: 1.0 ml min^{-1}; column temp.: 35°C; injection volume: 20 µl; λ detection: 213 nm. Isocratic elution was performed using 0.1% H_3PO_4 in water (v/v) (solvent A) and CH_3CN (solvent B) in 4:1 ratio (pH 2.17). The calibration curve was constructed by plotting peak areas of **1** against corresponding concentrations (10.0, 30.0, 50.0, 80.0, 110.0, 140.0, and 170.0 µg ml^{-1}). Five injections of each solution were used to obtain the curve. The experimental results were subjected to Huber test to reject the anomalous (Huber, 1998). The limits of detection and quantification were calculated according to IUPAC recommendations (Thompson et al., 2002).

Sample preparation

Aerial parts and roots were individually dried and ground. The materials (*ca.* 1.0 g) were extracted with CH_3OH (2 x 25 ml) or H_2O (2 x 25 ml) for 20 min by (i) magnetic stirring at room temp., (ii) magnetic stirring at 45°C, (iii) sonication at room temp., or (iv) sonication at 45°C. For the Soxhlet extraction (v) it was used 1.0 g of plant material and 75 ml of each solvent (CH_3OH or H_2O). Each type of extract was prepared in duplicate. After extraction, the solutions were individually filtrered and aliquots of 5 ml were separated for the quantification analyses by HPLC. Each aliquot was concentrated to dryness and the residue dissolved in 5 ml of CH_3OH for aerial parts extracts and in 3 ml for roots extracts. The samples were filtered through a 0.45 µm filter and 20 µl were injected, in triplicate, directly into HPLC.

Semi-quantitative HPLC analysis

HPLC analyses of the extracts were performed using 0.1% H_3PO_4 in water (solvent A) and CH_3CN (solvent B) as mobile phase, at a flow rate of 1.0 ml.min^{-1}. The following gradient was applied: 0-4 min, 20%B; 4-6 min, 20%-100%B; 6-11 min, 100%B. Detection wavelength was achieved at 213 nm. The identification of **1** in the samples was based on comparison of its retention time (t_R, 2.5 min) and UV-spectra with those of the standard compound.

Results and discussion

In this study, shikimic acid (**1**) is described for the first time in a species of the Pontederiaceae family. It was isolated and purified from the ethanol extract of *Eichhornia crassipes*