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Vapor-phase antifungal activity of *Ocimum micranthum* from Ecuadorian Amazon rainforest

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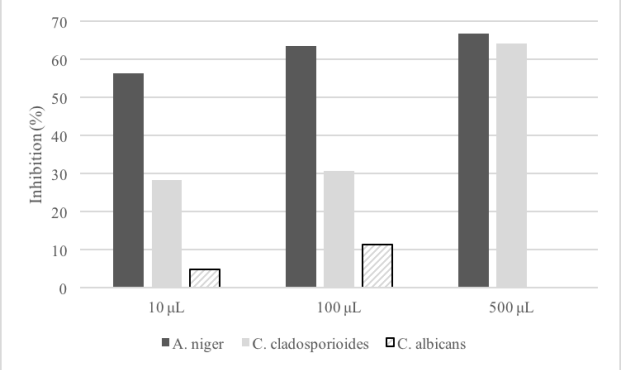
Graphical Abstract

Ocimum micranthum

Antifungal activity of *O. micranthum* VOCs



Abstract

Food grade chemicals are being investigated for their potential as preservative, in order to extend the spectrum of food preservation techniques. Protective atmosphere for food packaging purposes is one of the techniques gaining attention. The present study aimed at investigating the potential of Amazonian essential oil volatile organic components (VOCs) as preservative compounds in food industry. The antifungal activity of VOCs of Amazonian *Ocimum micranthum* essential oil was evaluated over three concentrations (10, 100, 500 µL/mL), through the vapor diffusion assay. The essential oil was tested against three fungi (*Aspergillus niger*, *Cladosporium cladosporioides*, *Candida albicans*); *Thymus vulgaris* essential oil has been used as positive control. PDA solidified medium was inoculated with fungal agar disks (0.5mm) on the center of Petri dishes. The EO was diluted in DMSO to obtain dilution. Then, 10 µL of each concentration was distributed on 10 mm sterile paper filter disks, then placed on the Petri dish cover, in order to spread the EO vapors. The Petri dishes were carefully closed with sealing tape. Fungi were stored at 27±2°C during 7 days and then colony diameters were measured. *A. niger* was the most sensitive at the highest concentration of EO (500 µL/mL), showing inhibition of 67%. *C. cladosporioides* followed with 64% inhibition, while *C. albicans* did not show any. These results concerning the vapor-phase antifungal activity of *O. micranthum* have been reported for the first time.

Introduction

A large number of food products needs protection against fungal spoilage to avoid deterioration during their shelf life. Several techniques as pulsed light, electric and magnetic fields or synthetic chemicals are used for this purpose [1]. However, awareness of consumers about safer food products increased demand for scientific studies aimed at find natural compounds able to preserve food during storage [2]. Food grade chemicals are being investigated for their potential as preservative, in order to extend the spectrum of food preservation techniques. Protective atmosphere for food packaging purposes is one of the techniques gaining momentum [1]. In this sense, volatile compounds (VOCs) of essential oils (EO) have been studied as a promise source of biologically active components for food conservation. EOs are volatile complex liquids extracted from different plant parts and they are usually used as flavors in food and cosmetics. However, their antimicrobial, antioxidant, antiviral and insecticidal properties are well-known [3] and recently it is increasing the interest on their volatile components [2]. Studies related to *in vitro* antimicrobial properties of EOs are mainly carried out through direct contact methods as disk or agar diffusion tests. However, there is lack of knowledge about antimicrobial activity of the vapor phase of EOs in order to set possible application uses. The present study aimed at investigating the potential of Amazonian essential oil volatile organic components (VOCs) of the species *Ocimum micranthum* Willd. as source of preservative compounds in food industry.

Materials and Methods

The essential oil of *O. micranthum* was obtained by a 2-hour hydrodistillation of fresh leaves, using a Clevenger apparatus. The oil was stored in amber vials at 4°C and previously dried over anhydrous sodium sulphate. The chemical characterization of the oil was performed through gas chromatography and gas chromatography-mass spectrometry analyses and the relative peak areas for each compound were averaged. The antifungal activity of Volatile Organic Components (VOCs) of Amazonian *Ocimum micranthum* essential oil was evaluated over three concentrations (10, 100, 500 μL/mL), through the vapor diffusion assay [1]. The essential oil was tested against the following three fungi: *Aspergillus niger*, *Cladosporium cladosporioides* and *Candida albicans*. *Thymus vulgaris* essential oil has been used as positive control considering its well-known antimicrobial properties [4]. PDA solidified medium was inoculated with fungal agar disks (0.5mm) on the center of Petri dishes. The EO was solubilized in dimethylsulfoxide (DMSO) to obtain dilutions. Then, 10 μL of each concentration was distributed on 10 mm sterile paper filter disks, and then placed on the Petri dish cover, in order to spread the EO vapors. Experiments were carried out in triplicate. The Petri dishes were carefully closed with sealing tape in order to avoid active component leaking. Blank was set by adding 10 μL of DMSO to the filter disks. Fungi were stored at 27±2°C during 7 days and then colony diameters were measured, in order to calculate the inhibition percentage based on the following formula: I(%)=[1-(Treated/Control)]x100.

Results and Discussion

O. micranthum distillation yield was of 0.68%. The essential oil was found to be rich in eugenol (18.6%), β-caryophyllene (17.0%), 1,8-cineole (11.4%), bicyclogermacrene (9%), cis-ocimene (8%) and α-caryophyllene (4.5%). Previous data reported by Trevisan et al. [5] also showed eugenol as the mayor compound but the amount was considerably higher (68,4%). *A. niger* was the fungus most sensitive to the essential oil. Inhibition was dose-dependent (10, 100, 500 μL/mL) and recorded values of 56.3%, 63,6% and 66.9% respectively. The fungus *C. cladosporioides* followed with 64% inhibition

at the EO concentration of 500 µL/mL, while *C. albicans* showed a very low inhibition at 10 and 100 µL/mL. A recent research performed by Caamal-Herrera et al. [6] on the essential oil of *O. micranthum*, showed a strong fungicide effect on *C. albicans* with minimal inhibitory concentration (MIC) of 125 µL/mL and a bacteriostatic effect against *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* (MIC 500, 250 and 500 µL/mL, respectively).

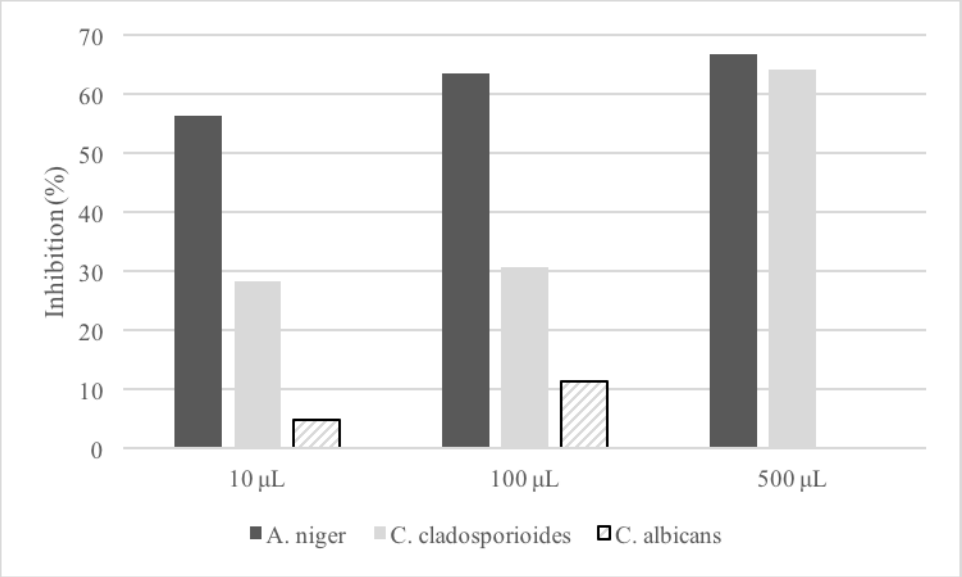


Figure 1 – Growth inhibition (%) of fungi caused by *O. micranthum* essential oil at 10, 100, 500 µL/mL concentrations

Conclusions

There are few studies concerning *O. micranthum* but this species deserves deepener investigation. In our research, for the first time, the volatile compounds of *O. micranthum* essential oil showed a dose-dependent antifungal activity especially against the foodborne fungus *A. niger*. These results, concerning the vapor-phase antifungal activity of *O. micranthum* essential oil, represent a good premise towards a healthier and eco-friendly conservation of food during storage stage. As future stages of research, a chemical characterization of the volatile components of the essential oil is suggested in order to known which compounds of vapor phase are directly involved in the antifungal activity.

Acknowledgments

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