EFFECT OF DRYING TIME ON THE ESSENTIAL OIL CONTENT OF *OCIMUM BASILICUM* L AND ITS ANTIMICROBIAL ACTIVITY

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Summary

The essential oil composition of *Ocimum basilicum* L. is very well established in the literature as well as its anti-microbial properties, however, the aim of this work is to correlate two different drying times and their influence in the anti-microbial activity. The sample that was let to dry longer showed the best anti-microbial activity against all strains tested except for *Staphylococcus aureus* strain.

Keywords: *Ocimum basilicum*, essential oil, anti-microbial activity, drying times.

Introduction

Ocimum basilicum L. is a species of Lamiaceae family, used all around the world as condiments in food preparation and also in the folk medicine for cough treatment, inflammation, dyspepsia, aches and pains[1]. This species is popularly known in Brazil as "alfavaca".

Essential oils are complex mixtures of volatile substances generally present in leaves at low concentrations. Before such substances can be analyzed, they have to be extracted from the matrix, using dried plant material. Several methods can be used for that purpose, e.g. hydro-distillation, steam distillation, Soxhlet extraction, and simultaneous distillation–extraction[2].

Ocimum basilicum's essential oil composition has already been established by gas chromatography and mass spectrometry (GC-MS) showing compounds like linalool, methylchavikol, methylcinnamate, linolene, eugenol and *t*-Cadinol[2], as well as its anti-microbial properties against different strains, including multidrug resistant ones isolates from the genera *Staphylococcus, Enterococcus, Pseudomonas*[1] and some inhibitory effect on *Aspergillus ochraceus*[3].

Although the composition of essential oil from this species has been much studied, it is also known that many factors can influence on it, such as harvest time, light absorption and soil type[4], but the interference caused by drying time is until now unknown. The aim of this work is to correlate two different drying times with the chemistry composition and its influence in the anti-microbial activity of the essential oil from *O. basilicum*.

Material and methods

Essential oil: *Ocimum basilicum* (Lamiaceae) was collected in Espírito Santo state (Brazil) in February 2004, identified by a specialist from "Herbário RFA" of the "Universidade Federal do Rio de Janeiro", Prof. Dr. Rosana Conrado Lopes.

Plant material was divided in two groups of 100 g each and dried at room temperature during ten and fifteen days.

After drying for ten days, the first group of *O. basilicum* was used to extract the essential oil by hydro-distillation using 750 mL of distillated water in a Clevenger-type apparatus, during 2 hours, with 1.5% yield. This essential oil was called "ALFAVACA 1".

Another plant material sample, dried for fifteen days had its essential oil extracted by the same way, during 2 hours, with 1.9% yield. The essential oil obtained in this second extraction was named "ALFAVACA 2".

Gas chromatography: Both essential oils were analyzed by GC-MS, under the same conditions, in order to determine chemical differences. The analyses were performed using a Hewlett Packard HP 6890 gas chromatograph fitted with a mass detector model 5973 and an automatic injector model 5683, both from Agilent Technologies. The mass spectra were obtained on 70 eV using PFK reference. The GC conditions used were a HP-5 column (30 m x 0.2 mm) was used, oven temperature was 60–240 ºC at 3 ºC min^{-1}, detector and injector temperatures were 240 $^{\circ}$C, injected volume was 1 mL. Carrier gas was Helium and total run time was 60 min.

Antimicrobial assay: "ALFAVACA 1" and "ALFAVACA 2" antimicrobial activities were determined by using the drop agar diffusion method described elsewhere[5]. The microorganisms tested were the fungi *Candida albicans* Serotype B ATCC 36802, *Cryptococcus neoformans* T_1-444 Serotype A (Universidade Federal de São Paulo, UNIFESP-SP), *Trichophyton rubrum* T544, *Fonsecaea pedrosoi* 5VPL (fungal collection from Hospital Clementino Fraga Filho, UFRJ) and the bacteria *Staphylococcus aureus* MRSA (BMB9393) (Hospital Clementino Fraga Filho, UFRJ); *Staphylococcus aureus* ATCC 25923. Microorganisms (2 x 10^5 cells) were spread over Petri plates containing BHI solid medium (Brain Heart Infusion) and after 10 minutes, a 10 μl drop of the essential oil diluted 1:1 with Tween 80 (0.5% in water) was placed in the center of each plate.

Likewise 10 μl of reference antibiotics (1mg/ml), used as positive control, were also tested: Amphotericin B, Methicillin and Vancomycin. All plates were incubated at 37°C and the time of incubation varied from 24 hours to 7 days, depending on the microorganism tested, after which the diameter of inhibition zone, in cm, was measured.

Results and discussion

Essential oil chemical composition: Both essential oil gas chromatograms indicate that the plant drying time does not interfere on qualitative chemical composition which seemed to be the same, although the content of "ALFAVACA 1" minor compounds increased in "ALFAVACA 2", showing that longer drying time doesn't change the type of metabolites, but concentrates them, mainly for the minor constituents. All the results obtained were compared with McLafferty's mass spectrum catalog[6] and the correlation between the calculated Kovats index with those published in the literature for each compound.

The major content in both essential oil set was the same, eucalyptol (RT = 11.7 min.). It was possible to identify linalool (RT = 14.9 min.), camphor (RT = 17.0 min.), ocimenol (RT = 19.2 min.) and isoeugenol (RT= 27.0 min.) as the main constituents in both essential oils. However, other compounds had increased concentration in "ALFAVACA 2" and they were β-*trans*-ocimene (RT = 7.7 min.), β-pinene (RT = 9.4 min.), β-myrcene (RT = 9.9 min.), 2-norbornanone (RT = 14.3 min.), (Z)-β-farnesene (RT = 29.8 min.), farnesene (RT = 30.4 min.), α-caryophyllene (RT = 31.2 min.), germacrene-D (RT = 32.4 min.), copaene (RT = 33.8 min.), and 1, 6-dimethyl-4-(methylethyl)-1-naphtalenol (RT = 39.0 min.).

Antimicrobial activity: The "ALFAVACA 2" essential oil leading to a higher anti-microbial effects against all strains tested, the exception being *Staphylococcus aureus* strain, which showed the same response for both essential oils. *O. basilicum* essential oil showed higher anti-fungal activity than anti-bacterial and the difference between both samples is higher against these strains too. The activities are shown on Table 1 as diameter of inhibition haloes (cm).

Table 1: Anti-fungal and anti-bacterial properties are shown as diameter of inhibition haloes (cm) using vancomicine for *S. aureus* and MRSA strains and anfotericine B for the others strains as positive control.

	Alfavaca 1	**Alfavaca 2**	**Control**
S.aureus	1.8	1.8	1.5
***S.aureus* (MRSA)**	1.5	1.8	1.6
F. pedrosoi	1.8	2.0	2.1
T. rubrum	3.3	4.5	1.5
C. albicans	2.5	3.5	1.8
C. neoformans	3.0	4.0	2.5

Conclusions

These results indicate the correlation between the drying time and the essential oil content and also the influence in the anti-fungal and anti-bacterial properties achieved, probably related to the enhancement of the other constituents in very small concentration in “ALFAVACA 1” essential oil, which appeared more concentrated in “ALFAVACA 2” essential oil. The difference between both essential oils was more noticed for anti-fungal property indicating that the compounds responsible for this activity are present as minor contents in essential oil.

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