

Inhibitory Action of Some Essential Oils and Phytochemicals on the Growth of Various Moulds Isolated From Foods

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ABSTRACT

The aim of this study was to determine the sensitivity profile of mould strains isolated from foods to some essential oils and phytochemicals. The assayed mould strains were: Fusarium spp., Rhizopus spp., Aspergillus flavus, A. niger and Penicillium spp. According to results, Lippia alba N.E. Brown, Peumus boldus Molina, Lippia microphylla Phil., Citrus limon Risso and Cymbopogon citratus Stapf. essential oil and the phytochemicals citral, eugenol and mircene showed prominent antimould activity. Among the products that evidenced antimould activity, citral and eugenol showed the lowest minimum inhibitory concentrations, which was 1% and 4%, respectively, for the most of the tested mould strains.

Key words: Plant products, antimould activity, sensitivity profile

INTRODUCTION

Moulds are opportunistic biological agents of ubiquitous nature. They are able to colonize diverse substrates including foods. Because of their powerful arsenal of hydrolytic enzymes, these microorganisms can cause a high degree of deterioration when present in/on foods and can be responsible for considerable economic losses. Furthermore, they can act as potential producer of toxic metabolites, named mycotoxins, which are potentially damaging to consumers health.

It is well established that some plants contain compounds able to inhibit the microbial growth (Naqui et al., 1994). These plant compounds can have different structures and different action when compared with antimicrobials conventionally used

to control the microbial growth and survival (Nascimento et al., 2000). The potential antimicrobial properties of plants had been related to their ability to synthesize, by the secondary metabolism, several chemical compounds of relatively complex structures with antimicrobial activity, including alkaloids, flavonoids, isoflavonoids, tannins, coumarins, glycosides, terpenes, phenylpropanes, organic acids (Nychas, 1996).

The wide and indiscriminate use of chemical preservatives has been cause of the appearance of over and over resistant microorganisms, leading for occurrence of emerging food borne diseases (Gibbons, 1992; Kaur and Arora, 1999; Akinpelu, 2001). Due to this, there is an increasing interest to obtain alternative antimicrobial agents to use in

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food conservation systems. One of the main procedures used in the research of biologically active substances is the systematic screening of the interaction between microorganisms and plant products. This procedure has been source of useful agents to control the microbial survival in different microbiology application fields (Salvat et al., 2001). Plant products of recognized antimicrobial spectrum could appear in food conservation systems as main antimicrobial compound or as adjuvant to improve the action of other antimicrobial compounds (Kaur and Arora, 1999). The aim of this study was to evaluate the sensitivity profile of moulds strains isolated from foods to some essential oils and phytochemicals with emphasis for a possible future use of the ones as alternative antimould compounds.

MATERIALS AND METHODS

Essential oils and phytochemicals

Essential oils evaluated for their antimould spectrum were extracted from *Cimbopogon citratus* Stapf., Poaceae family; *Citrus limon* Risso, Rutaceae family, *Eucalyptus globulus* Labill., Mirtaceae family; *Eugenia uniflora* L., Mirtaceae family; *Lippia alba* N.E. Brown, Verbenaceae family; *L. microphylla* Phil., Verbenaceae family; and *Peumus boldus* Molina, Lamiaceae family. Phytochemicals evaluated for their antimould spectrum were cariofilene, citral, eucalyptol, eugenol and mircene. Essential oils were provided by the Laboratory of Pharmaceutical Technology, Department of Pharmaceutical Sciences, Federal University of Paraíba, Brazil and the phytochemicals were provided by the Laboratory of Phamacotechnique, Department of Pharmaceutical Sciences, State University of Ponta Grossa, Brazil. These essential oils and phytochemicals were extracted according to standard procedure described by Matos (1994).

Mould strains

The mould strains were isolated from farinaceous food samples (cassava flour and corn flour) marketed in João Pessoa city, Brazil. The microscopic characteristic observation technique using laminated microculture was used as procedure for mould identification (Lacaz et al., 1991). *Aspergillus flavus*, *A. niger*, *Fusarium* spp., *Penicillium* spp. and *Rhizopus* spp. strains were

isolated, identified and used as test microorganisms in the antimould assays.

Screening for antimould activity

Diffusion plate procedure using filter paper discs was used for the screening for antimould activity of essential oils and phytochemicals (Odds, 1989; Hadacek and Greger, 2000). For this, 1mL of mould suspension (approximately 10^6 spores) prepared with sterile 0.85% physiological saline solution was uniformly spread on the sterile Sabouraud agar Petri dishes. Sterile filter paper discs (Whatman n. 1, diameter 6mm) were soaked with 0.02mL of each essential oil or phytochemical and placed on the center of Sabouraud agar Petri dishes inoculated with the mould suspension. The incubation time was 7-10 days at 25°C. At the end of the incubation period, the inhibition halo diameters were measured using calipers and expressed in millimeters. When the inhibition halo observed was equal or higher than 10mm diameter, it was considered as positive antimould activity (Lima et al., 1993).

Determination of the Minimum Inhibitory Concentration (MIC)

Essential oils and phytochemicals that presented antimould activity in the screening assay were evaluated for their MIC determination and it was carried out by the plate diffusion procedure using wells in dishes (Hadacek and Greger, 2000). For this, 1mL of mould suspension (approximately 10^6 spores) prepared with sterile 0.85% physiological saline solution was uniformly spread in the sterile Sabouraud agar Petri dishes. After inoculum absorption by Sabouraud agar, wells were made using sterile glass stems (diameter 6mm) which were filled with 50µL of solutions of the essential oil or phytochemical. The different concentration solutions used in the MIC assay were obtained as follow: 0.4mL of essential oil or phytochemical and 0.04mL of Tween 80 were taken in sterile tubes and the volume was completed to 5mL using distilled sterile water. This mixture was shaken for 5 minutes using Vortex and serial dilutions were made to obtain solutions with final concentrations of 8.0, 4.0, 2.0, 1.0 and 0.5%. The incubation period was 7-10 days at 25°C. The concentration able to develop inhibition halos equal or higher than 10mm diameter was considered as MIC. Control was carried out with Tween 80 (Lima et al., 1993) by the diffusion technique using filter

paper discs (Cecon, diameter 6mm) (Bauer and Kirby, 1966).

RESULTS AND DISCUSSION

Essential oils are volatile substances contained in several plant organs. They play several essential functions for the plant survival including defense against invader microorganisms (Cowan, 1999). Screening results for essential oils antimould activity are shown in Table 1. From all essential oils evaluated only one (*E. uniflora* essential oil) did not show any antimould effect. In the screening assay, six essential oils (*C. limon*, *C. citratus*, *E. globulus*, *L. alba*, *L. microphylla* and *P. boldus*) presented inhibitory action on at least two assayed mould strains. On the other hand, in the MIC determination assay those essential oils did not present any activity to the tested concentration (8.0, 4.0, 2.0, 1.0 and 0.5%) on the mould strains (data not shown). Particularly, only *L. alba* essential oil was able to exert antimould activity on *A. niger* up to 4.0% concentration. Contrary to our results, some researches have shown that this essential oil presented a low antifungal activity and a high antibacterial activity (Lemos et al., 1990; Lima, 1996). *C. limon* essential oil (Rutaceae family) showed inhibitory activity on *A. flavus*, *Fusarium* spp. and *A. niger* (Table 1). Antimicrobial activity of Rutaceae family species have been observed on dermatophytes (Lima, 1996) and on some moulds (Megalla et al., 1980). *C. citratus* essential oil

showed activity against *Rhizopus* spp., *Fusarium* spp. and *A. flavus* (Table 1). Some moulds as *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Curvularia*, *Fusarium* and *Rhizopus* isolated from stored foods were sensitive to the action of this essential oil (Dubey and Mishra, 1993).

P. boldus essential oil was active against *A. niger*, *A. flavus* and *Fusarium* spp. In other surveys, this essential oil has shown antimicrobial spectrum when in biological interactions with yeasts (*Candida*, *Tricophyton*, *Microsporum* and *Epidermophyton*) and bacteria (*Staphylococcus aureus*, *S. epidermidis* and *Escherichia coli*) (Lima, 1996; Farias and Lima, 2000).

The essential oils evaluated in this work have a great variety of phytochemicals that could be considered as responsible for a larger or smaller antimicrobial activity, as follow: i) E-citral, Z-citral, α -pinene, geraniol, mircene and 1- β pinene in *C. citratus* essential oil; ii) eucalyptol, canfene, mircene, p-methane, terpinen, citral, citronelal in *E. globulus* essential oil; iii) furanodiene, elemene, 2- β pinene, terpen, linaleol, cariofilene in *E. uniflora* essential oil; iv) x-elemene, pinene, cis-cariofilene, carvacrol, timol and terpineol in *L. alba* essential oil; and v) cineole, terpinene, α -pinene, eugenol, cedrol in *P. boldus* essential oil (Craveiro et al., 1981; Lavabre, 1992).

Screening results for antimould activity of the phytochemicals on the moulds strains are shown in Table 2. Cariofilene and eucalyptol did not present any activity on the assayed moulds strains.

Table 1 - Medium values of the inhibition halos (mm) presented in the screening for antimould activity of essential oils on moulds strains isolated from cassava flour and corn flour.

Mould strains	Essential oils ^a							Control ^b
	<i>C. limon</i>	<i>C. citratus</i>	<i>E. globulus</i>	<i>E. uniflora</i>	<i>L. alba</i>	<i>L. microphylla</i>	<i>P. boldus</i>	
<i>Penicillium</i> spp.	0	0	0	0	12	0	7	0
<i>A. niger</i>	10	0	12	0	24	24	35	0
<i>A. flavus</i>	13	10	0	0	0	0	13	0
<i>Fusarium</i> spp.	0	12	0	0	10	10	10	0
<i>Rhizopus</i> spp.	12	13	13	0	12	12	0	0

^a absolute concentration; ^b control: Tween 80 (absolute concentration)

Citral, eugenol and mircene presented antimould activity and among all citral showed best results. Mircene in all tested concentrations in the MIC assay was not able to inhibit the growth of *Fusarium* spp. and *Rhizopus* spp.

MIC of citral and eugenol are shown in Table 3. Citral presented MIC of 0.5% for *A. niger*, *A. flavus* and *Fusarium* spp., 2.0% for *Penicillium* spp. and 8.0% for *Rhizopus* spp. Eugenol presented a MIC of 2.0% for *A. flavus* and 4.0%

for *Penicillium* spp., *Fusarium* spp. and *Rhizopus* spp. *A. niger* was the single mould strain that presented resistance to eugenol on all tested concentrations.

Phytochemicals are chemical substances characterized as organic biomolecules found and isolated from different plant derivative products, such as teas, decocts, infusions, extracts and essential oils. These compounds are responsible for the biological activities exerted by several plants and their derivative products (Brull and Coote, 1999). Cariofilene, citral, eucalyptol, eugenol and mircene are volatile substances present in several essential oils extracted from plants with recognized antimicrobial properties (Craveiro et al., 1981; Lavabre, 1992; Lima et al., 1993). Thymol, eugenol, anethole, menthol, citral, pinenes, isothiocyanates, cinnamaldheydes, carvacrol, carvone, benzoic acids, fenolic acids, flavones are some phytochemicals that have been studied regarding their antimicrobial spectrum on some bacterial and fungi species (Megalla et al., 1980; Gnananickman and Mansfield, 1981;

Odebyi et al., 1984; Karapnan, 1990; Delaquis and Mazza, 1995; Helander et al., 1998; Nascimento et al., 2000). In general, the inhibitory action of natural products on mould cells involves cytoplasm granulation, cytoplasmic membrane rupturing and inactivation and/or synthesis inhibition of intercellular and extracellular enzymes. These actions can occur of isolate or concomitant way and culminate with the mycelium germination inhibition (Cowan, 1999).

Occurrence of phytochemicals (Table 2) that did not show antimould activity (cariofilene and eucalyptol), could suggest that probably in some situations certain phytochemicals have their antimicrobial action shown only when acting in synergistic way with other constituents; in other words, when they are in form of extracts, essential oils, decocts, etc. Moreover, resistance and invasion capability of the mould strains used as test microorganisms could be cited as important interfering factors to the antimould efficiency of the essential oils and phytochemicals included in the antimould assays.

Table 2 - Medium values of the inhibition halos (mm) presented in the screening for antimould activity of phytochemicals on moulds strains isolated from cassava and corn flour.

Mould strains	Phytochemicals*					Control**
	Cariofilene	Citral	Eucalyptol	Eugenol	Mircene	
<i>Penicillium</i> spp.	0	37	0	15	0	0
<i>A. niger</i>	0	20	0	0	0	0
<i>A. flavus</i>	0	30	0	25	0	0
<i>Fusarium</i> spp.	0	42	0	10	12	0
<i>Rhizopus</i> spp.	0	35	0	40	11	0

* absolute concentration; ** Tween 80 (absolute concentration)

Table 3 - MIC values of citral and eugenol on moulds strains isolated from cassava flour and corn flour.

Mould strains	Phytochemicals MIC (%)	
	Citral	Eugenol
<i>Penicillium</i> spp.	2.0	4.0
<i>A. flavus</i>	0.5	2.0
<i>A. niger</i>	0.5	- *
<i>Fusarium</i> spp.	0.5	4.0
<i>Rhizopus</i> spp.	8.0	4.0

(-) sensitivity absence to all analyzed concentrations

The results obtained justify future researches emphasizing the antimicrobial properties of plant products and their possible use as viable alternatives to control the microbial growth in foods.

CONCLUSIONS

According to the results shown we could say that: i) majority of the essential oils and phytochemicals tested showed some “in vitro” inhibitory activity against moulds, having prominence *Lippia alba*

essential oil, citral and eugenol; ii) the plant derivative products could be used as alternatives to control microbial contaminants of foods such as moulds, and could be able to become useful tools for application in foods conservation systems.

RESUMO

O objetivo deste estudo foi determinar o perfil de sensibilidade de cepas de fungos filamentosos isolados de alimento a alguns óleos essenciais e fitoconstituintes. As cepas fúngicas utilizadas nos ensaios antimicrobianos foram: *Fusarium* spp., *Rhizopus* spp., *Aspergillus flavus*, *A. niger* e *Penicillium* spp. De acordo com os resultados obtidos, os óleos essenciais de *L. Alba* N.R. Brown, *P. boldus* Molina, *L. microphylla* Phill, *C. limon* Risso e *C. citratus* Stapf. e os fitoconstituintes citral, eugenol e mirceno mostraram destacada atividade antifúngica. Dentre os produtos que apresentaram atividade antifúngica, o citral e eugenol mostraram as menores CIM's, as quais foram 1% e 4%, respectivamente, para a maioria das cepas fúngicas testadas.

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