



Study of the cytotoxic and antifungal activity of the essential oil of *Elyonurus muticus* against *Candida* spp.

Liliana I. Sabini¹, Paula Cordero Gabrielli¹, Cristina V. Torres¹, Franco Matías Escobar¹, Mario Cacciabue¹, Marisa Rovera¹ and Nicolás Kolb²

¹Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta 36, Km. 601, Río Cuarto, Provincia de Córdoba, Argentina.

²Laboratorio Central, Facultad de Ciencias Exactas, Químicas y Naturales de la Universidad Nacional de Misiones, Misiones, Argentina. e-mail: lsabini@exa.unrc.edu.ar

INTRODUCTION

During the last years an increment of fungal infections has been observed all over the world. Those caused by yeasts, mainly *Candida* spp, are the most frequent in immunodepressed hosts, especially in those HIV infected, and in patients treated with immunosuppressors or antibiotics of wide spectrum.

For the treatment of these pathologies, the availability of effective and low toxic antifungal agents is scarce. This situation and the appearance of strains resistant to these agents (Sanglard and Odds, 2002) make necessary the search of new antifungals, being the natural sources an excellent option according to WHO suggestions.

Elyonurus muticus ('Espartillo' or 'Aibé') is a predominant aromatic species in northeastern Argentine that shows essential oils (EO) with different chemical composition according to the collection place, called 'chemotypes' or 'chemical races'. Their biotechnical application has impact in perfumery.

At present studies are not known on its phytomedicinal properties, but taken into account the relative abundance of the plant species is that complementary studies on antimicrobial actions were designed, bringing out in these assays the evaluation of the antifungal capacity, associated to the cytotoxic action on eucariote cells of three EO chemotypes of *E. muticus*.

METHODOLOGY

Three EO chemotypes: acorenone, citral and geraniol, were provided by Ing. Nicolás Kolb (Universidad Nacional de Misiones, Misiones, Argentina).

Cytotoxicity assays were carried out in triplicate

on monolayers of Vero cells, treated with different concentrations of each EO. The Maximum Non-Cytotoxic Concentration (MNCC) was determined after 72 h incubation at 37°C by direct observation under optic microscope.

The antifungal capacity was evaluated by the microdilution technique in broth: M27-A according the National Committee of Clinical Laboratories Standards (NCCLS) recommendations, (Cantón Lacasa *et al.*, 2001).

Six strains of *Candida* spp isolated from human clinical cases compatible to candidiasis were used (Table 1). The value of Minimum Inhibitory Concentration (MIC) was determined, which allowed evaluating the Minimum Fungal Concentration (MFC) defined as the minimum concentration at which less than 0.1% of the initial inoculum survived. All assays were carried out in duplicate.

Table 1. Origin of different *Candida* spp strains isolated from human clinical cases.

Strain	<i>Candida</i> spp					
	1	2	3	4	5	6
Origin	UT	UT	V	RT	V	V

UT : Urinary tract; RT: Respiratory tract; V: Vagina.

RESULTS AND DISCUSSION

All EO of *E. muticus* showed to be active as inhibitors of fungal development, in variable ranges against the tested strains of *Candida* spp. The positivity criterion was adopted from the direct visualization, using the optic microscope, of the wells containing the strain problem and the EO, determining the presence or not of microbial colonies (Fig. 1). The diameter of the

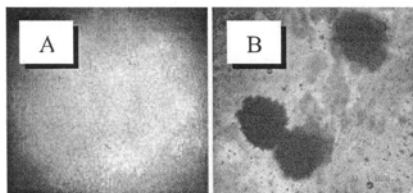


Figure 1. Strain 5 (A) treated with geraniol EO at 0.87 mg/ml, (B) treated with acorenone EO at a concentration of 1.94 mg/ml

The MIC values obtained for the 3 chemotypes are shown in Table 2. Geraniol was the most active since it only required 0.87 mg/ml to inhibit the development of 2 of the 6 studied strains (33.33%). On the contrary, acorenone was the less active chemotype of the 3, and required 17 times more concentration to inhibit the fungal strains, and even for strain 2 were needed 71 times more than this chemotype to achieve the same inhibitory effect. It is worth to mention that all strains were sensitive to the used conventional antifungal agents (nistatine and amphotericine B).

Table 2. MIC values with 100% inhibition of the different EO chemotypes of *E. muticus*. ND: not determined.

EO CHEMOTYPES of <i>E. muticus</i> used at different concentrations (mg/ml)			
Strain	Acorenone	Citral	Geraniol
1	15.55	3.72	1.74
2	62.18	ND	1.74
3	31.09	3.72	0.87
4	15.55	3.72	1.74
5	15.55	3.72	1.74
6	15.55	3.72	0.87

It was also observed that the strains from similar clinical cases didn't evidence to be equally sensitive to the same one chemotype, suggesting that the intrinsic features to the fungal species or of the patient that had suffered the

values of MFC and MIC for citral and geraniol chemotypes, their fungicide power was demonstrated. Regarding cytotoxicity, the MNCC values were 0.106 mg/ml, 0.165 mg/ml and 0.370 mg/ml for acorenone, citral and geraniol chemotypes, respectively. These values demonstrated that the EO of *E. muticus* possess a marked toxic capacity since the MNCC values exceeded the MIC.

The toxicity induced by the 3 EO was evidenced as morphological alterations of the cellular substrate, with tissue disorganization and its later loosening (Fig. 2).

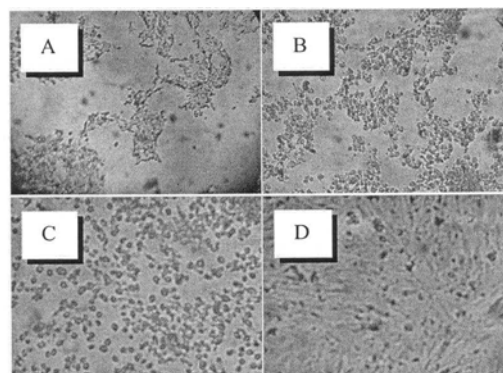


Figure 2. (A, B and C) Damages induced by citral EO chemotype in Ver0 cells at toxic concentrations. (D) Cell control.

CONCLUSIONS

Although the EO of *E. muticus* shows activity against different strains of *Candida* spp, a marked cytotoxic activity is evidenced at high concentrations of different biotypes. Therefore, this study allows to assert that the EO lacks a selective toxicity, which determines that further investigations are required about the possible reversion of this toxicity.

These studies highlight the ethnobotanic potential of the species *Elyonurus muticus*, since the fungicide capacity of its essential oils transforms them into potential disinfectants, applicable on inert surfaces.

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on



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