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RESEARCH ARTICLE

DETERMINATION OF THE ANTIFUNGAL CAPACITY OF TOTAL EXTRACTS OF *Sinapis alba* L BY THE METHOD OF PLATES AND WELLS

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ABSTRACT

Sinapis alba is a plant of the cruciferous family, known as white mustard and for its use as a condiment, also for the therapeutic effect of its seeds. This project was proposed to determine the antifungal capacity relative to *S. alba* extracts using three solvents with different polarities, apolar petroleum ether, moderately polar dichloromethane and polar ethanol, against fungi: *Penicillium chrysogenum, Aspergillus niger, Candida albicans* and *Saccharomyces cerevisiae* by the plates and wells method. The results allowed to determine that the total extracts of the flowers had relative antifungal activity on *A. niger* using the solvents petroleum ether and ethanol using a volume of 50uL, however, no other extract caused the inhibition of the fungi used in the study.

INTRODUCTION

Sinapis alba is a plant of the Cruciferous family, its Latin genus "Sinapis" means mustard, the Latin species "alba" means white, refers to the light color of the seeds. It is also known as *Brassica alba, Brassica hirta* or yellow mustard. The seeds are considered diuretic, emetic, expectorant, stimulant. It is believed that the plant even has emollient and sedative properties (J. Duke *et al.,* 1983). The method used for extraction of plant organs (flowers, leaves, stems, roots) of *S. alba* was soxhlet (A. Patiño *et al.,*2000), a liquid solid extraction consisting in contacting a solid previously crushed and macerated with a liquid (solvent), it is usually helped with agitation, temperature or ultrasound for greater efficiency. The yeasts *S. cerevisiae,* is known to produce ethanol in high yield even at high concentrations of sugar and alcohol (Y. Lee *et al.,*2017), and *C. albicans* which is part of the oral and vaginal microbiota; is the main cause of nosocomial fungemias, seriously affecting immune suppressed patients (Y. Tong *et al.,* 2017). Filamentous fungi play an important role in industrial biotechnology because of its wide use for the production of compounds such as antibiotics, metabolites and enzymes such as P. chrysogenum producer of B-lactams (F. Polli *et al.,* 2016) and *A. niger* lipases producer (T. Utami *et al*, 2017), cellulolytic enzymes (T. Carvalho *et al.,* 2011) and citric acid (I-U. Haq *et al.,* 2003), also causing nosocomial infections by his low virulence (E. Atchade *et al.,* 2017). The objective of this article was to verify that the extracts of *Sinapis alba* (root, stem, leaves, flowers) have relative antifungal activity against *Saccharomyces cerevisiae, Candida albicans*, *Penicillium chrysogenum* and *Aspergillus niger,* analyzed by the plate and well method.

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MATERIALS AND METHODS

Obtaining strains

The microorganisms used in the study are strains of fungus culture collection of the Pontificia Javeriana University in Bogotá, Colombia.

Culture Media

DIBIO TSA was prepared in distilled water in a concentration of 40 g / L, subsequently was sterilized at 121 ° C and 103.421 Pa (15 psi).

Inoculum Preparation

A suspension of each fungus was made in TSA broth (10% of the volume of TSA agar to be used), these suspensions were incubated at 28 ° C for seven days. At the end of the

incubation time dilutions of the inoculum were made, They were seeded in depth and incubated under the same conditions to verify the initial concentration that was mixed with the agar TSA outside 10^6UFC / mL.

Extracts

The extraction process was carried out separately from the stem, root, leaves and flowers of the plant, using soxhlet type extraction, using as solvents petroleum ether, ethanol and dichloromethane. A suspension of 100 mg of each extract in 1 mL of DMSO was performed for testing of antifungal activity.

Preparation of agar plates

Approximately 50 mL of medium inoculated in each 100x15mm petri dish was served, after the solidification of the agar was refrigerated for 24h. After cooling the medium, a central perforation was made with an inverted pipette of 5mL and sealed with 30 uL of sterile culture medium, finally 50 or 100 uL of each extract was added in triplicate.

Controls

The inoculated medium without perforation was used as growth control of each fungus, to perform the white control 50 and 100uL of DMSO will be used in duplicate, and a commercial antifungal as positive control (5.25% sodium hypochlorite).

Incubation

Yeasts and filamentous fungi were incubated at 28 ° C for 7 days.

Measurement of relative antimicrobial activity

After the incubation, the measurement of the halos of each assembly was performed and the relative antimicrobial activity with respect to the commercial antifungal and DMSO was calculated using the following formula (L. Ramírez *et al.,* 2007).

$$\%Inhibition = \frac{extract\ halo - white\ halo}{positive\ control\ halo - white\ halo} \times 100$$

Diameter of the white halo (mm): DMSO
Diameter of the control (mm): 5.25% sodium hypochlorite

RESULTS

Total extracts obtained from flowers in solvents: petroleum ether, ethanol and dichloromethane

The yeasts *S. cerevisiae* and *C. albicans* did not present halos of inhibition with respect to the total extracts of the flowers of Sinapis alba, nor did they present against dimethylsulfoxide; however, they showed inhibition against the positive control carried out with hypochlorite. Filamentous fungi P. chrysogenum and *A. niger* showed inhibition against dimethyl sulfoxide, however, *A. niger* was the only one to show inhibition against petroleum ether and ethanol extracts, presenting a greater halo than the positive control performed. Particularly in the case of *A. niger* against the petroleum ether solvent in the volume of 100uL a false positive could be reported, since the relative antifungal activity would be given by the DMSO and not by the composition of the plant material; On the contrary, in ethanol and petroleum ether solvents with a volume of 50uL, the inhibition is greater than in the target, which indicates that these extracts have a possible action against this fungus (Tabla 1).

Table 1. Measurement results of the halos (mm) and the percent inhibition of total extracts from flowers against four microorganisms and their respective controls

Microorganismo	Extracto (Flores)	Volúmen (uL)	Halos			% Inhibición
			halo de inhibición (mm)	halo de inhibición control con DMSO (mm)	halo de inhibición control de hipoclorito (mm)	
Saccharomyce scerevisiae	Dicloro	50	-	-	16	0
		100	-			0
	Etanol	50	-			0
		100	-			0
	petróleo	50	-			0
		100	-			0
Candida albicans	Dicloro	50	-	-	15	0
		100	-			0
	Etanol	50	-			0
		100	-			0
	petróleo	50	-			0
		100	-			0
Aspergillus niger	Dicloro	50	-	18	12	0
		100	-			0
	Etanol	50	31			-216,66667
		100	-			0
	petróleo	50	21			-50
		100	13			0
Penicillium chrysogenum	Dicloro	50	-	16	15	0
		100	-			0
	Etanol	50	-			0
		100	-			0
	petróleo	50	-			0
		100	-			0

Total extracts obtained from stems and leaves in solvents: petroleum ether, ethanol and dichloromethane

The yeasts *S. cerevisiae* and *C. albicans* showed no inhibition halos on total extracts of *S. alba* leaves or stems, nor against

dimethyl sulfoxide; however, they showed inhibition against the positive control carried out with hypochlorite. Filamentous fungi *P. chrysogenum* and *A. niger* presented haloes against dimethyl sulfoxide and the control with hypochlorite, but not against Sinapis alba total extracts from leaves or stems.

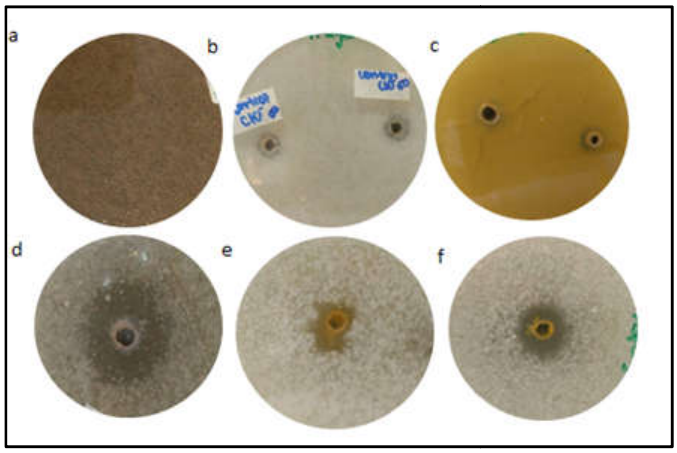

Figure 1. Results and controls for test *A. niger* vs. flowers. a. growth control b. control with hypochlorite (12 mm) c. Controls with DMSO (18 mm) d. 50uL ethanol (31 mm) e. 100uL petrol (13 mm) f. 50uL petrol (21mm)

Total extracts obtained from the root in solvents: petroleum ether, ethanol and dichloromethane

The yeasts *S. cerevisiae* and *C. albicans* showed no inhibition halos on total extracts of *S. alba* root, nor against dimethyl sulfoxide; however, they showed inhibition against the positive control carried out with hypochlorite.

Filamentous fungi *P. chrysogenum* and *A. niger* showed inhibition against dimethyl sulfoxide and control with hypochlorite, but not against total extracts of this plant organ.

DISCUSSION

Sinapis alba is reported in the literature as a plant producing peptides with antifungal action, tannins and defensins, the latter were classified in 1995 for causing morphological changes in the hyphae or not doing while they had antifungal effect (W. Broekaert *et al.,* 1995). The production of these peptides according to the literature is carried out on plant organs leaves, roots, stems and flowers (S. Meira *et al.,* 2013), however, the only organ that when using polar solvents (ethanol) and nonpolar (petroleum ether) showed antifungal activity were flowers, possibly because there are a greater concentration or concentration sufficient to inhibit the growth of *A. niger* as it is reported in literature (S. Meira *et al.,* 2013). *S. cerevisiae* yeast is reported in literature as sensitive to tannins and defensins produced by *S. alba* (S. Meira *et al.,* 2013), but was not inhibited by the total extracts of any of the plant organs, possibly because they were in low concentration. The organs leaves, stem and root did not show antifungal activity on any of the fungi, they could have a lower concentration of the peptides than in the flowers, making them incapable of causing an inhibitory effect on the fungi.

Brassica oleracea, also belonging to the cruciferous family, is reported in literature for inhibiting the growth of *C. albicans* and *A. niger* by approximately 50% from a juice made by centrifuging and homogenizing its leaves; this plant is glucosinolates producer (M. Sisti *et al.,* 2003), oils produced during secondary metabolism of plants belonging to this family (G. Sanchez *et al.,* 2015). There are reports of glucosinolate production in *S. alba* (A. Ekanayake *et al.,* 2012) and as noted in literature these compounds must be in a high concentration to inhibit microorganisms (M. Sisti *et al.,* 2003), However, previous studies have shown that fungi respond differently to a stimulus, such as facing a plant extract, causing mycelial inhibition and sporulation inhibition (F. Espinosa *et al.,* 1991). *P. chrysogenum* did not sporulate during any of the trials, probably because of some volatile compounds (C. Linton *et al.,* 1973) which caused sporulation inhibition, possibly from plant extracts; however, A. niger sporulated and its mycelial growth was not inhibited against the extracts of plant organs: leaves, stems and roots, but when faced with the ethanol and petroleum ether extracts from flowers, its mycelial growth and sporulation was inhibited. at the same time (Figure 1), this is consistent with an earlier study by exposing A. flavus and A. parasiticus to *Larrea tridentata* extracts, causing a reaction in the fungus to inhibit mycelial growth and sporulation (C. Sanchez *et al.,* 2000). There are reports of the inhibitory action of S. alba against phytopathogenic fungi such as *Alternaria brassicae* (S. Pedras *et al.,*1997), *Alternaria brassicicola* (M. Mazumder *et al.,*2013) and *Verticillium dahliae* (C. Alcántara *et al.,* 2009) so the use of extracts in agricultural industries, green houses, etc. could be investigated to control the dissemination of these fungi by the white crops, because the experiments carried out by the researchers involve the whole plant and not extracts of the vegetable organs.

DMSO is a hygroscopic and colorless liquid, used as solvent, of aprotic nature that is associated with cations, leaving the anions free and reactive (H. Beyer *et al.,*1987), had an inhibitory effect on the filamentous fungi and not against the yeasts by the differences in their cell wall, in filamentous fungi *P. chrysogenum* and *A. niger* is composed mostly of chitin and glucan, different from the yeast containing mainly mannan and glucan, also chitin but in a smaller amount (B. García *et al.,*1968), Chitin could be the weak point of the cell wall that caused the sensitivity of these fungi.

Conclusion and Recommendations

The vegetal organ of *Sinapis alba* with relative antifungal activity was the flowers, in the solvents petroleum ether and ethanol with volume of 50 uL on the microorganism *Aspergillus niger*, using the plates and wells method. It is recommended to filter the extracts to favor their conservation, eliminate contamination with other microorganisms and use them in a sterile environment to perform microbiological tests.

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