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**ORIGINAL ARTICLE**

# *Candida albicans* PROTEIN PROFILE CHANGES IN RESPONSE TO THE BUTANOLIC EXTRACT OF *Sapindus saponaria* L.

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## SUMMARY

*Candida albicans* is an opportunistic human pathogen that is capable of causing superficial and systemic infections in immunocompromised patients. Extracts of *Sapindus saponari*a have been used as antimicrobial agents against various organisms. In the present study, we used a combination of two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) to identify the changes in protein abundance of *C. albicans* after exposure to the minimal inhibitory concentration (MIC) and sub-minimal inhibitory concentration (sub-MIC) of the butanolic extract (BUTE) of *S. saponaria* and also to fluconazole. A total of six different proteins with greater than 1.5 fold induction or repression relative to the untreated control cells were identified among the three treatments. In general, proteins/enzymes involved with the glycolysis (GPM1, ENO1, FBA1), amino acid metabolism (ILV5, PDC11) and protein synthesis (ASC1) pathways were detected. In conclusion, our findings reveal antifungal-induced changes in protein abundance of *C. albicans*. By using the previously identified components of the BUTE of *S. saponaria* (e.g., saponins and sesquiterpene oligoglycosides), it will be possible to compare the behavior of compounds with unknown mechanisms of action, and this knowledge will help to focus the subsequent biochemical work aimed at defining the effects of these compounds.

**KEYWORDS:** *Candida albicans*; *Sapindus saponaria;* Two-dimensional gel electrophoresis; Saponins; Mass spectrometry.

## INTRODUCTION

*Candida albicans* is an opportunistic yeast that causes different forms of candidiasis in human hosts. Normally, *C. albicans* acts as a commensal organism in the gastrointestinal and genitourinary tracts; it can be isolated from approximately 70% of the population. Nevertheless, it can lead to disease, typically in immunocompromised and neutropenic patients[1]. Several antifungal agents have been used to treat *Candida* infections, but the effectiveness of many of these fungicides is still being evaluated. The most effective drugs for treating *Candida* infections are the azoles, polyenes and echinocandins. Natural compounds are a source of many active compounds that show multiple therapeutic effects[2], and because the existing antifungals can have some toxicity, natural compounds have attracted attention.

Species from the Sapindaceae family are known for their traditional medicinal use as diuretics, stimulants, expectorants, natural surfactants, sedatives, and vermifuges and for their use in the treatment of stomachaches and dermatitis in many parts of the world[3]. *Sapindus saponaria* L. (Sapindaceae), popularly known as "sabão-de-soldado" and "saboeiro", is a medium-sized deciduous tree that occurs in the tropics of the Americas and India, where the fruit is used as a soap and as a medicine against ulcers, scabies, joint pain, inflammation[4,5,6,7] and skin lesions caused by fungi[8]. *Sapindus saponaria* is a potential candidate for the treatment of candidiasis *in vitro* and *in vivo*[9,10,11].

In a recent study, members of our research group isolated and identified the main constituents of the n-BuOH extract (BUTE) of the pericarps of *S. saponaria*: two acetylated triterpene saponins, S1 and S2, and also an acyclic oligoglycoside (OGASA-01). The same group also demonstrated an excellent inhibitory *in vitro* activity of BUTE against *Candida albicans* and non-*C. albicans* isolated from patients with vulvovaginal candidiasis (VVC)[11], indicating that this plant may be used as an antifungal agent for this pathology. In general, saponins have shown antifungal activity against *C. glabrata*, *C. albicans*, *Trichosporon beigelii*, *Penicillium avellaneum*, *Pyricularia oryzae*, *Cryptococcus neoformans*, *Coccidioides immitis*, and *Saccharomyces cerevisiae*, as well as against the dermatophytes *Microsporum canis* and *Trichophyton*

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*mentagrophytes*[12,13,14]. Damke *et al*.[9] showed that *S. saponaria* BUTE has antifungal activity *in vivo* using Wistar rats infected with azole-susceptible *C. albicans* (SCA), azole-resistant *C. albicans* (RCA), and azole-resistant *C. glabrata* (RCG). According to Francis *et al*.[10], the main mechanism for the antifungal activity of the saponins is their interaction with steroids of the fungal membrane.

Proteomics has been used to identify many proteins of *Candida albicans* under different conditions, such as adaptive responses to ambient pH, salt, cadmium and peroxide stress, the formation of biofilms and adaptive responses to antifungal agents[15,16,17,18]. The identification of these proteins is important because they can become targets for the development of novel therapeutic agents.

In this study, we used a combination of two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) to identify the changes in protein abundance of *C. albicans* after exposure to the butanolic extract (BUTE) of *Sapindus saponaria* and to the commercial antifungal fluconazole. Despite speculation about the possible mechanisms of action of the saponins, there were no prior studies that demonstrated protein changes after *Candida albicans* exposure to *S. saponaria* BUTE.

## MATERIAL AND METHODS

**Antifungal agents:** For the proteomic experiments, the butanolic extract (BUTE) of *Sapindus saponaria* was prepared at the Laboratory of Pharmacology of UEM as described below, and a stock solution was diluted in Milli-Q water at 18 mg/mL. The stock solution of fluconazole was prepared immediately before use in 100% DMSO at 500 mg/mL and stored at -20 °C.

**Plant and components:** Dry fruit pericarps of *S. saponaria* were collected on the campus of the State University of *Maringá, Paraná*, Brazil (UEM). The plant was identified by staff members of the UEM Department of Botany, and an exsiccate was deposited in the Herbarium of this institution (HUM 11710). The dried pericarps of the fruits (450.0 g) of *S. saponaria* were ground and extracted with EtOH: $H_2O$ (9:1) at room temperature by a process of dynamic maceration with constant mechanical stirring. The extraction was carried out in an amber flask at ambient temperature for 6 h per day on six consecutive days. The extract was concentrated under low pressure in a rotary evaporator at a temperature of 40 °C. After the solvent was eliminated, the extract was frozen in liquid nitrogen and lyophilized in a Martin Christ Alpha 1-2 freeze dryer. The lyophilized extract was stored in a closed plastic flask and kept frozen.

**Test of antifungal susceptibility:** Drug susceptibility assays were carried out through the microdilution method with some adaptations for natural products[19]; fluconazole (FLU) (Pfizer Inc., New York, NY, USA) was dissolved in broth, and the lyophilized n-BuOH extract (BUTE) was dissolved in sterile distilled water to obtain a 10 mg/mL solution of each agent. For proteomic assays, the antifungal susceptibility test used an initial BUTE concentration of 18 mg/mL. The tests were carried out in triplicate in sterile plastic microplates (TPP Zellkultur TestPlate 96F, Switzerland).

Stock solutions of FLU were prepared at 10 times the final concentration and diluted in RPMI-1640 with L-glutamine, bicarbonate-free, supplemented with 2% dextrose and buffered to pH 7.0 with 0.165 M of 3-(N-morpholino) propanesulfonic acid (MOPS). Each well of the microplate received an increasing concentration of FLU ranging from 0.125 to 64 µg/mL.

Regarding the BUTE test, 100-µL aliquots of RPMI were added to columns 2 to 11. Then, 100- µL aliquots of BUTE were added to columns 1 and 2 of the microplates. From column 2 onward, 1:2 serial dilutions were performed to achieve maximal dilutions of 1:1,024.

For each isolate, negative (only RPMI), positive (RPMI plus inoculum, without antifungal addition), and diluent (alcohol and inoculum) controls were included on the plates. All the assays were carried out in triplicate. The treated microplates were incubated at 35 °C for 48 h. The results of the fluconazole tests were determined in a microplate reader (Asys Hitech GmbH, Eugendorf/Austria), and the BUTE tests were evaluated by visual comparison using mirror reflex.

The MIC of FLU was defined as the first column with a significant growth reduction (~50%) when compared to the growth of the positive control. Concerning the BUTE, the results of the MIC were determined as the lowest concentration that was capable of inhibiting 100% of the yeast growth compared to the corresponding positive control. The $MIC_{50}$ and $MIC_{90}$ for FLU and BUTE were defined as the MIC capable of inhibiting 50% and 90% of the isolate, respectively.

**Scanning electron microscopy for the study of *S. saponaria* BUTE effect on *C. albicans* cells:** Scanning electron microscopy was performed to verify the effect of *S. saponaria* BUTE on cell morphology of *C. albicans*. Aliquots of 1 mL of *C. albicans* ATCC 90028 cells were collected after 2 and 6 h of contact with 281.24 µg/mL of the extract, and the cells were prepared for scanning electron microscopy (SEM). This *S. saponaria* extract concentration corresponds to the MIC of *S. saponaria* BUTE observed for the inoculum size used for this assay. At the 6-h time point, cells were harvested and prepared for the proteomic assay. For the SEM, cells from 1 mL of culture were recovered by brief centrifugation and fixed in 2.5% glutaraldehyde for 2 h. The wells were washed twice with 0.1 M cacodylate and gradually dehydrated in ethanol. After coating with gold, the samples were examined with a Shimadzu SS-550 Superscan electron microscope.

**Organism and culture conditions for the proteome assay:** *C. albicans* ATCC 90028 was cultured overnight in 20 mL of YPD broth (1% yeast extract, 2% peptone, 1% dextrose) in a temperature-controlled incubator at 30 °C. The cultures were diluted in 500 mL of fresh YPD medium to an optical density (OD) of 0.1 at 600 nm and subsequently grown at 30 °C until an OD of 0.2 was reached. After growth to an OD of 0.2, the cells were treated with 750 µg/mL fluconazole diluted in 0.5% DMSO, or 140.62 µg/mL or 281.24 µg/mL of the BUTE of *S. saponaria*, corresponding to the minimal sub-inhibitory (sub-MIC) and inhibitory concentrations (MIC), respectively. Control experiments were performed using 0.5% DMSO (the solvent for the fluconazole) added to the culture, and all cultures were kept at 30 °C in a shaker incubator at 250 rpm (Nova Ética 430/RDBP) in a temperature-controlled incubator for 6 h. After the 6-h incubation, the ODs were measured.

**Preparation of the protein extracts:** Approximately 100 mg of *C. albicans* cells was collected by centrifugation in conical tubes for 5 min

at 4500 rpm (Hettich® Rotina 420). The pellet of cells was washed twice with ice-cold PBS buffer and once with ultrapure water. The cells were re-suspended in 500 µL of 7 M urea, 2 M thiourea, 4% CHAPS and 50 mM DTT buffer containing 1x Protease Inhibitor Mix (GE Healthcare Bio-Science AB, Piscataway, MA, USA), and the suspension was sonicated on ice (30 bursts of 10 seconds ON, 30 seconds OFF with 40% amplitude). The lysates were clarified by centrifugation at 14,000 rpm (Eppendorf® 5430 R) for 20 min at 4 °C, and the protein concentrations were determined using Bradford assays[21] and BSA as a standard.

**Isoelectric focusing and 2DE analysis:** Proteins were prepared for isoelectric focusing using the 2D Clean-Up kit (GE Healthcare Bio-Science AB, Piscataway, MA, USA) and re-suspended in DeStreak Rehydration Solution (GE Healthcare). The protein concentration was verified by the Bradford assay and adjusted to 250 µg in 250 µL of DeStreak Rehydration Solution with 0.5% IPG pH 3-10 buffer. The samples were applied to isoelectric focusing (IEF) strips (3-10 pH range, 13 cm; GE Healthcare), which were overlaid with 0.6 mL DryStrip Cover Fluid (GE Healthcare) and rehydrated overnight at room temperature. Isoelectric focusing was performed using an IPGphor II IEF System (GE Healthcare Bio-Science AB, Uppsala, Sweden) at 20 °C, 500 V for 1 h, 1,000 V for 1 h, 8,000 V for 2.5 h, and 8,000 V for 30 min.

Thereafter, the strips were equilibrated for 15 min with gentle shaking in a solution of 6 M urea, 2% SDS, 30% glycerol, 75 mM Tris-HCl pH 8.8, 0.002% bromophenol blue, and 1% DTT. The strips were then equilibrated for 15 min in a second buffer containing 2.5% iodoacetamide instead of DTT.

The equilibrated strips were overlaid on homogeneous 12% (1.0 mm thick) SDS-PAGE gels, electrophoresed at 100 V/ 10 mA/ 4 W per gel for 15 min and then 300 V/ 50 mA/ 60 W for approximately 4 h at 15 °C using a Hoefer SE 600 Ruby power supply (GE Healthcare Bio-Science AB, Uppsala, Sweden) with 12 µL of ColorPlus Prestained Protein Ladder (New England, Biolabs, Beverly, MA, USA). The proteins were detected by a modified Neuhoff Blue Silver Coomassie Colloidal Blue staining protocol[22] and maintained in 5% acetic acid. The gels were scanned at an optical resolution of 300 dpi on a high-resolution image scanner (GE Healthcare, USA), and the images were analyzed using the ImageMaster Platinum 2D v6.0 software (GE Healthcare, USA). The differentially expressed proteins were selected for identification.

**Protein Identification via In-gel Digestion and Mass Spectrometry:** Protein spots were excised from the 2DE gels and subjected to in-gel digestion with sequencing-grade trypsin (Promega, USA) as described by Shevchenko[23] with minor modifications; the digested proteins were then analyzed using mass spectrometry. After incubation at 37 °C for 16 h, aliquots of each hydrolyzed sample were mixed with a saturated matrix solution of a-cyano-4-hydroxycinnamic acid, spotted onto MALDI target plates and allowed to air dry. Mass spectra (MS) were acquired using a MALDI-TOF/TOF Autoflex II spectrometer (Bruker Daltonics, Germany) with the MASCOT search engine (Matrix Science Ltd., UK) against the "Fungi" subset of the SwissProt database (http://www.expasy.ch/sprot/) or NCBInr (http://www.ncbi.nlm.nih.gov/).

## RESULTS

**Morphological features of *C. albicans* after exposure to *S. saponaria* BUTE:** SEM was performed to observe general morphological changes in *C. albicans* cells after exposure to *S. saponaria* BUTE (Fig. 1). Comparisons were made between the control *C. albicans* cells (untreated) and the *C. albicans* cells treated with a concentration of 281.24 µg/mL of BUTE. The untreated-cells were generally smooth-walled bodies after both 2 h and 6 h of culture (Fig. 1A and 1B). The BUTE-treated cells presented surface irregularities, including convolutions (Fig. 1C and 1D).

**Comparison of the effects of fluconazole and the MIC and sub-MIC of *S. saponaria* BUTE on the protein expression pattern of *C. albicans***

Changes in the *C. albicans* proteome were analyzed by comparing cells treated with the MIC and sub-MIC of *S. saponaria* BUTE and cells treated with fluconazole with untreated control cells. The minimum inhibitory concentration (MIC) of *S. saponaria* BUTE (281.24 µg/mL) was determined at 0.2 O.D. The high concentration of fluconazole, 750 µg/mL, is due to a large amount of inoculum and takes into account the conditions observed by Bruneau *et al*.[24]. Among the three conditions, a total of six proteins, not including individual protein isoforms, that had greater than 1.5-fold induction or repression relative to their amounts in untreated control cells were identified by in gel enzymatic digestion and MALDI-TOF-TOF (Fig. 2). The molecular proprieties and changes in the relative levels of expression are shown in Table 1, and the putative biological functions of these proteins are shown in Table 2. In general, the proteins are involved in glycolysis and amino acid metabolism.

**Proteins that are responsive for the MIC of *S. saponaria* BUTE:** The six proteins that were responsive for the MIC of BUTE of *S. saponaria* based on MALDI-TOF-TOF analysis are displayed in Fig. 2B. Among these, two proteins were induced, and four proteins were repressed. The induced proteins were guanine nucleotide-binding protein subunit beta-like protein (ACS1) and likely mitochondrial ketol-acid reductoisomerase (ILV5). The expression of fructose-bisphosphate aldolase (FBA1), pyruvate decarboxylase (PDC11), enolase 1 (ENO1), and phosphoglycerate mutase (GPM1) was reduced.

**Proteins that are responsive for the sub-MIC of *S. saponaria* BUTE:** The six proteins that were responsive to the sub-MIC of *S. saponaria* BUTE based on MALDI-TOF-TOF analysis are displayed in Fig. 2C. Among them, four proteins were induced, and 2 proteins were repressed. The induced proteins were guanine nucleotide-binding protein subunit beta-like protein (ACS1), phosphoglycerate mutase (GPM1), peroxisomal catalase (CTA1) and fructose-bisphosphate aldolase (FBA1). The expression of likely mitochondrial ketol-acid (ILV5) and fructose-bisphosphate aldolase (FBA1) was decreased.

**Fluconazole-responsive proteins:** The four proteins that were responsive to fluconazole according to MALDI-TOF-TOF analysis are displayed in Fig. 2D. Among them, one protein was induced, and three proteins were repressed. The induced protein was peroxisomal catalase (CTA1). The expression of fructose-bisphosphate aldolase (FBA1), pyruvate decarboxylase (PDC11) and phosphoglycerate mutase (GPM1) was decreased.

## DISCUSSION

In the present study, we used a combination of 2D-PAGE and MALDI-TOF mass spectrometry to verify changes in protein expression

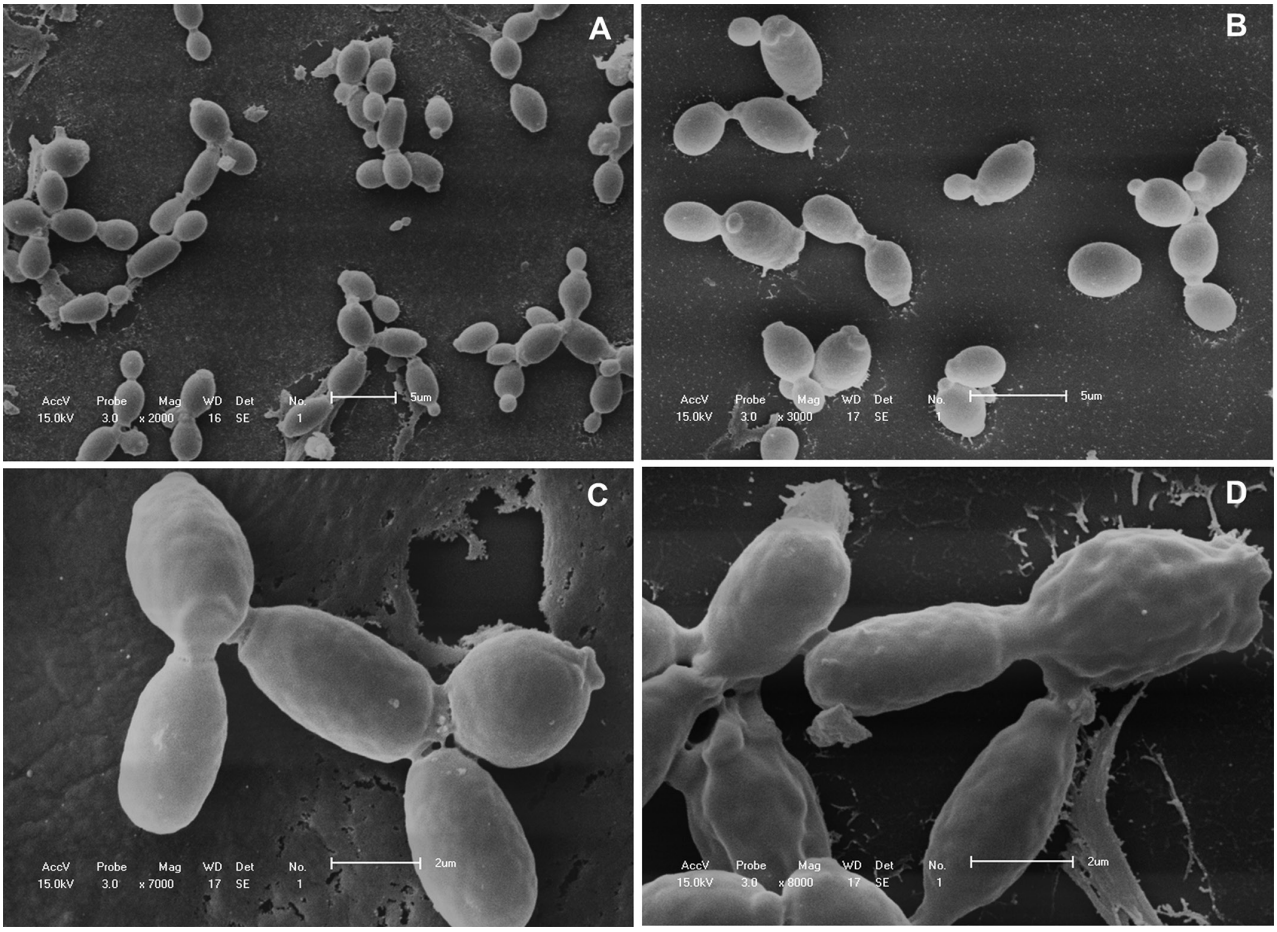


**Fig. 1** - Scanning electron microscopy of *C. albicans* cells cultured for 2 h (A) and 6 h (B) without treatment (control) and for 2 h (C) and 6 h (D) in the presence of 281.24 µg/mL of *S. saponaria* BUTE. The majority of BUTE treated cells are distorted and have irregular surfaces.

of *C. albicans* cells exposed to three treatments (MIC and sub-MIC of BUTE of *S. saponaria* and fluconazole). Proteins/ enzymes involved in glycolysis (GPM1, ENO1, FBA1), amino acid metabolism (ILV5, PDC11) and protein synthesis (ASC1) pathways were detected. It is difficult to obtain a clear interpretation of the mechanism of action of *S. saponaria* BUTE from this preliminary study of proteome changes. However, some observations can be made. Several of the biological processes are associated with carbohydrate metabolism, as observed by Martínez-Gomariz *et al*.[25]. Comparing the effects of the two *S. saponaria* BUTE concentrations on protein expression, we observed that more proteins had a decreased abundance after treatment with the MIC than with the sub-MIC. These included mainly proteins involved in glycolysis. Some proteins appeared as multiple spots on the gels, such as fructose-bisphosphate aldolase, pyruvate decarboxylase and phosphoglycerate mutase. The variation in the spots specifically occurs in the pI values. This may be caused by post-translational modifications such as phosphorylation. However, we cannot rule out that some of the spots may be due to the experimental procedure; this was observed for spot number 11, related to fructose-bisphosphate aldolase, which was found to be the same protein in spots 3 and 5 but with different pIs and MWs.

The ASC1 protein (guanine nucleotide-binding protein subunit beta-like protein) was found to be upregulated in *C. albicans* after exposure to both the MIC and sub-MIC of *S. saponaria* BUTE, with increased values of 2.51 and 1.60 fold, respectively. Located at the head of the 40S ribosomal subunit, in the vicinity of the mRNA exit channel, ASC1 serves as a scaffold protein that can recruit other proteins to the ribosome. This protein is also a signal transducer that plays a pivotal role in cellular adhesion and virulence by regulating specific gene expression in *C. albicans.* It is also repressed in the stationary phase[26] and presents a role in the induction of filamentation and biofilm formation[27]. This protein had no differential expression in cells treated with fluconazole. Given that fluconazole is a fungistatic antifungal and that *C. albicans* cells enter a stationary phase when they stop growing, ASC1 may be repressed due to fluconazole (data not shown).

Enzymes involved in the glycolytic pathway such as FBA1 (fructose-bisphosphate aldolase), PDC11 (pyruvate decarboxylase) and GPM1 (phosphoglycerate mutase) were found to be downregulated in *C. albicans* cells treated with both the MIC of *S. saponaria* BUTE and fluconazole. ENO1 (enolase) was downregulated only in *C. albicans* cells treated with fluconazole. MALDI-TOF analysis of the sub-MIC-treated cell proteins showed three possible isoforms of FBA1; two of these isoforms were downregulated, and the other was upregulated. This protein is involved in a reversible reaction that is required for both glycolysis and gluconeogenesis. It is known that different antifungal agents have varying

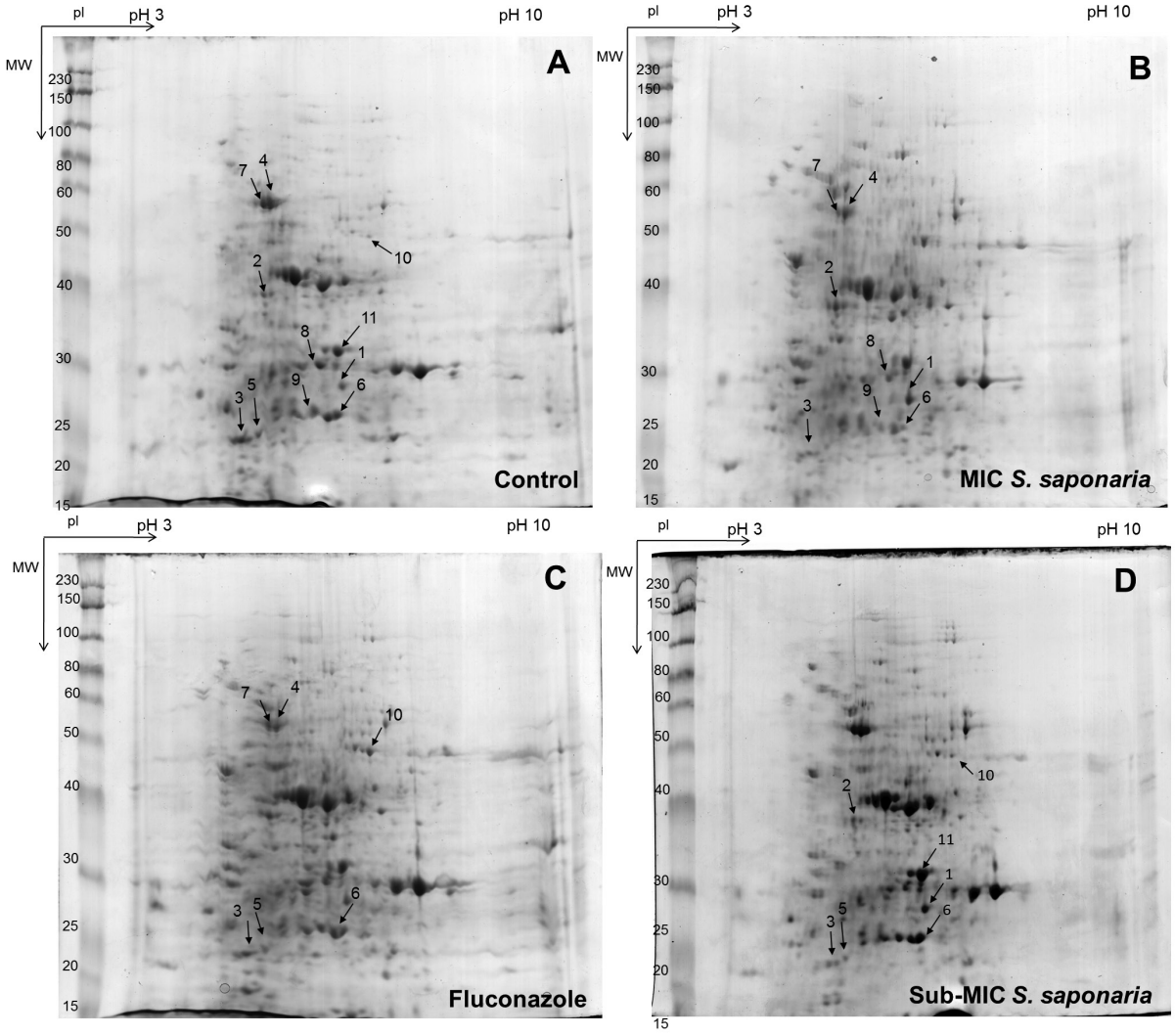


**Fig. 2** - 2-D expression profiles of proteins from *C. albicans* ATCC 90028. (A) Untreated control, (B) treated with the MIC of *S. saponaria* BUTE, (C) fluconazole-treated, (D) treated with the sub-MIC of *S. saponaria* BUTE. Equal amounts of protein (250 µg) were loaded and separated on 13-cm IPG strips (pH 3–10), followed by electrophoresis on 12% SDS-PAGE gels in second-dimension electrophoresis. The gels were stained by Silver Coomassie Colloidal Blue.

effects on the expression of GPM1 in *C. albicans*. Hoehamer *et al*.[16] have shown that GPM1 mRNA increases during fluconazole exposure but is downregulated when cells are exposed to ketoconazole. One reasonable cause of the decreased expression of GPM1 in *C. albicans* cells exposed to fluconazole may be the growth phase of the harvested cells (the stationary phase). GPM1 mRNA levels increase during the exponential growth phase in yeast and then decrease to relatively low levels in the stationary phase. Additionally, the MIC of *S. saponaria* BUTE used in this work may have had a deleterious effect on *C. albicans* strains, preventing their growth and decreasing GPM1 expression.

Shirtliff *et al*.[28] used proteomic analysis to demonstrate that several glycolytic enzymes (e.g., glyceraldehyde 3-phosphate dehydrogenase, enolase, phosphoglycerate mutase and pyruvate kinase) were downregulated when *C. albicans* was exposed to farnesol, a natural 15-carbon organic compound and acyclic sesquiterpene alcohol that is used by *C. albicans* as a quorum-sensing molecule, inhibiting filamentation. *S. saponaria* BUTE also contains sesquiterpenes, and one possible effect of this component may be the downregulation of these glycolytic enzymes. However, this interpretation remains speculative because we were unable to test the saponins and sesquiterpenes of *S. saponaria* BUTE separately.

The protein ILV5 (likely mitochondrial ketol-acid reductoisomerase) had a differential expression in *C. albicans* after exposure to the MIC and sub-MIC of *S. saponaria* BUTE, with 2.09-fold upregulation and 1.60-fold downregulation, respectively, compared to the untreated control cells. Ketol-acid reductoisomerase is described as an antigenic; GlcNAc, amino acid starvation (3-AT) induced; macrophage-repressed protein that is present in the exponential and stationary phases[26,29,30.31].

Peroxisomal catalase (CTA1) increased in abundance in *C. albicans*

**Table 1**
List of *Sapindus saponaria* and Fluconazole responsive proteins identified by MALDI-TOF mass spectrometry (difference at least 1.5-fold)

| Spot no. | MS/MS Score | MS score | Protein coverage % | Fold change[1] | Gel isoform | pI | Mr | Protein ID |
|---|---|---|---|---|---|---|---|---|
| **MIC *S. saponaria*** | | | | | | | | |
| Upregulated Proteins | | | | | | | | |
| 1 | Nd | 162 | 45 | 2.51 | | 6.07 | 34533.62 | ASC1 |
| 2 | 48 | 199 | 48/37 | 2.09 | | 6.17 | 44935 | ILV5 |
| Downregulated Proteins | | | | | | | | |
| 3 | 28 | 149 | 5/32 | 5.10 | 1 | 5.69 | 48/37 | FBA1 |
| 4 | Nd | 162 | 21 | 1.82 | 1 | 5.39 | 62401.92 | PDC11 |
| 5 | Nd | 168 | 34 | 2.46 | 2 | 5.69 | 39190.76 | FBA1 |
| 6 | 26 | 82 | 4/19 | 4.06 | 1 | 5.79 | 27437.46 | GPM1 |
| 7 | 5 | 312 | 2/43 | 3.10 | 2 | 5.39 | 62401.92 | PDC11 |
| 8 | 43 | 159 | 3/26 | 1.93 | | 5.54 | 47202.50 | ENO1 |
| 9 | 22 | 136 | 4/28 | 1.60 | 2 | 5.79 | 27437.46 | GPM1 |
| **Sub-MIC *S. saponaria*** | | | | | | | | |
| Upregulated Proteins | | | | | | | | |
| 1 | Nd | 162 | 45 | 1.60 | | 6.07 | 34533.62 | ASC1 |
| 6 | 26 | 82 | 4/19 | 1.84 | | 5.79 | 27437.46 | GPM1 |
| 10 | 51 | 115 | 1/21 | 2.05 | | 6.03 | 55029.13 | CTA1 |
| 11 | Nd | 252 | 53 | 1.87 | 1 | 5.69 | 39190.76 | FBA1 |
| Downregulated Proteins | | | | | | | | |
| 2 | 48 | 199 | 48/37 | 1.60 | | 6.17 | 44935 | ILV5 |
| 3 | 28 | 149 | 5/32 | 4.13 | 2 | 5.69 | 39190.76 | FBA1 |
| 5 | Nd | 168 | 34 | 2.72 | 3 | 5.69 | 39190.76 | FBA1 |
| Fluconazole | | | | | | | | |
| Upregulated Proteins | | | | | | | | |
| 10 | 51 | 115 | 1/21 | 4.87 | | 6.03 | 55029.13 | CTA1 |
| Downregulated Proteins | | | | | | | | |
| 3 | 28 | 149 | 5/32 | 2.15 | 1 | 5.69 | 39190.76 | FBA1 |
| 5 | Nd | 168 | 34 | 2.58 | 2 | 5.69 | 39190.76 | FBA1 |
| 4 | Nd | 162 | 21 | 3.35 | 1 | 5.39 | 62401.92 | PDC11 |
| 7 | 5 | 312 | 2/43 | 1.88 | 2 | 5.39 | 62401.92 | PDC11 |
| 6 | 26 | 82 | 4/19 | 1.60 | | 5.79 | 27437.46 | GPM1 |

[1]Fold change: Change in expression between treated cells and control cells. Nd: Not done. The spot numbers are those indicated on Fig. 2.

exposed to the sub-MIC of *S. saponaria* BUTE and fluconazole, but the MIC did not cause differential expression of this protein. It has been reported that CTA1 plays a role in resistance to oxidative stress[32], virulence, core stress responses, and other functions that are regulated by fluconazole.

In conclusion, our findings reveal antifungal-induced changes in protein abundance of *C. albicans*. By using the previously identified components of the *S. saponaria* BUTE, such as saponins and sesquiterpene oligoglycosides, it will be possible to compare the behavior of compounds with unknown mechanisms of action, which will help to focus subsequent biochemical studies. It is possible that some of these responsive proteins may represent potential targets for the development of novel therapeutic agents against this pathogenic fungus.

**Table 2**
Function of the proteins exhibiting a greater than 1.5-fold induction and repression which were identified by means of in-gel enzymatic digestion and MALDI-TOF-TOF

| Protein ID | SwissProt or NCBI Accession number | Metabolic process | Function |
|---|---|---|---|
| ASC1 | P83774 | Ribosomal protein | A component of the 40S ribosomal subunit and a signal transducer |
| ILV5 | gi\|68483473 | Branched-chain amino acid biosynthetic process | Involved in branched chain amino acid biosynthesis. |
| FBA1 | Q9URB4 | Glycolysis | Catalyzes the aldol condensation of dihydroxyacetone phosphate with glyceraldehyde 3-phosphate to form fructose 1,6-bisphosphate in gluconeogenesis and the reverse reaction in glycolysis. |
| PDC11 | P83779 | Glycolysis | Catalyses the decarboxylation of pyruvic acid to acetaldehyde and carbon dioxide. |
| ENO1 | P30575 | Glycolysis | Catalyzes conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis and the reverse reaction during gluconeogenesis. |
| GPM1 | P82612 | Glycolysis | Catalyses conversion of 2-phospho-D-glycerate to 3-phospho-D-glycerate. |

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## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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