TITLE: *IN VITRO* INHIBITION OF *Candida albicans* GROWTH BY POLYPHENOL-RICH EXTRACT OF *Hibiscus sabdariffa*

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ABSTRACT:

Candidiasis is currently considered one of the most common fungal infections that affect humans. The disease is caused by *Candida* spp. yeasts that inhabit human oral mucosa in healthy individuals. *Hibiscus sabdariffa* is a worldwide plant used traditionally as medicinal for several purpose. Recent investigations have demonstrated the antimicrobial potential of the species. This work aimed to evaluate the antifungal activity of *H. sabdariffa* against *C. albicans* and to determine the phytochemical characteristics of HECi. The extract of *H. sabdariffa* was prepared by sequential solvent extraction in a Soxhlet apparatus. The polar extract was used in this study in order to obtain a polyphenol-rich extract. Phenol composition of the polyphenol-rich extract of *H. sabdariffa* (PRE-Hs) was evaluated by preliminary phytochemical screening, thin layer chromatography (TLC), high performance liquid chromatography and by quantitative assay (total flavonoid content and total phenol content). A broth microdilution method was used to determine the antifungal activity of PRE-Hs against *Candida albicans* ATCC 10231. After 24h of incubation of five concentrations of the extract (62,5-1000 µg/mL) with *C. albicans* inoculum (10^2-10^3 cells/mL), 20 µL of a MTT solution (5 mg/mL) was added to each well. Then, the absorbance of each well was measured at 492 nm in a microplate reader after additionally 24h of incubation with MTT. The results are expressed as inhibition growth percentual (%inhibition). The preliminary phytochemical screening of the PRE-HS was regarded positive for all the phenolic substances classes tested (tannins, organic acids and flavonoids). Liquid chromatography analysis was able to identify the presence of several flavonoids in the extract (retention time = 2-5 min., maximum absorbance peaks at 254-265 nm and 344-369). High amounts of phenol content and flavonoid were found in the extract (TPC = 152,40 ± 14,18 mg of gallic acid equivalents/g of PRE-HS and TFC = 28,57 ± 1,60 mg quercetin equivalents/g of PRE-HS). After 48h of incubation, all concentrations of the extract were able to inhibit the growth of *C. albicans* with maximum cytotoxic activity observed at 62,5 µg/mL (35,93±0,75% of inhibition). The PRE-Hs was shown to possess antifungal activity against *C. albicans* and this activity may be related to its chemical composition rich in phenol constituents.

Keywords: *Candida albicans*, *Hibiscus sabdariffa*, Polyphenols, Broth microdilution, MTT.

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