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Antifungal Activity of Ester Derivatives from Caffeic Acid against *Candida* Species



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ABSTRACT

Objective: This study evaluated the *in vitro* antifungal activity of a series of five ester derivatives of the caffeic acid on different *Candida* species. **Methods:** The antifungal assay was carried out by the microdilution method in 96-well microplates for determining the minimal inhibitory concentrations (MICs). **Results:** The results obtained in this study revealed that most of the evaluated compounds presented weak to moderate antifungal effect. Among the five evaluated ester derivatives, the methyl caffeate (1) was found to exhibit the best antifungal activity, with MIC values of 512 µg/ml against all strains of *C. albicans*, *C. tropicalis* and *C. krusei*. Further, all tested strains showed precisely the same degree of sensitivity to screened compounds. Some structural characteristics of the tested ester derivatives that may affect the antifungal activity (alkyl ester side chain length) were observed, giving information on possible functional groups important for the antifungal effect. **Conclusion:** The findings suggest that future research adding new derivatives from caffeic acid may be interesting in the search for new antifungal compounds.



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INTRODUCTION

Fungal infections are generally among the most common cause of several skin diseases in developing countries [1]. Opportunistic fungal infections, principally resulting from the species of *Candida*, *Cryptococcus* and *Aspergillus* are life-threatening in immunocompromised individuals, mainly HIV-infected patients and those receiving immunosuppressive chemotherapies for cancer and organ transplantation[2]. Due to the increasing number of individuals of this category, fungal diseases have increased in the last two decades, affecting millions of people globally and causing an estimated 1.5 million deaths each year [3].

Candida species are recognized as the most commonly fungi involved in the etiology of the mycotic infections [4]. Candidiasis is the fungal infection most common, being *C. albicans* the most pathogenic and prevalent etiologic agent. Further, other *Candida* species, such as *C. parapsilosis*, *C. krusei* and *C. tropicalis* are also causative agents of candidiasis [5,6].

The last decade has watched a considerable augmentation in the prevalence of resistance to antimicrobial agents, presenting important implications for morbidity, mortality and healthcare in the community. So, there is a pressing need to discover and develop new classes of antifungal compounds for the treatment of fungal infections, and the research on natural products derived compounds has increased and highlighted in recent years due to their key role in drug discovery[7,8]. For example, some studies published in the literature have related the antifungal activity of different cinnamic acid derivatives against fungi species[9-12].

Thus, the main aim of the current work was to evaluate the antifungal activity of a series of five ester derivatives of the caffeic acid against three strains of *Candida albicans* (*C. albicans* ATCC-76645, *C. albicans* LM-120 and *C. albicans* LM-106), two strains of *C. krusei* (*C. krusei* LM-08 and *C. krusei* LM-13) and three strains of *C. tropicalis* (*C. tropicalis* ATCC-13803, *C. tropicalis* M-14 and *C. tropicalis* M-7A).

CHEMISTRY

Purification of the compounds were performed by column chromatography on silica gel 60, ART 7734 MERCK using solvent gradient Hex: EtOAc confirmed by analytical thin layer chromatography on silica gel 60 F₂₅₄, revealing ultraviolet light at two wavelengths (254 and 366 nm) using a Mineralight apparatus or H₂SO₄ in 5% ethanol. FTIR spectra were recorded

in an FTIR spectrometer IR Prestige-21-Shimadzu model using KBr pellets. ^1H and ^{13}C NMR spectra were obtained in Varian MERCURY machines (200 and 50 MHz for ^1H and ^{13}C , respectively). Deuterated solvent was used (DMSO- d_6). Tetramethylsilane (TMS) was used as the internal standard. Chemical shifts (δ) were measured in parts per million (ppm) and coupling constants (J) in Hz.

Methodology

General procedure for the synthesis of compounds

A mixture of caffeic acid (0.25g) and alcohol (50 ml) was heated under reflux in presence of sulphuric acid (0.4 ml) until the completion of its reaction (5-8 hours), which was checked by a single spot in TLC. Then, alcohol was removed under reduced pressure and the solution was diluted with 20 ml of water. The product was extracted with ethyl acetate (15 ml). The organic phase was neutralized successively with NaHCO_3 5% and water, dried over anhydrous Na_2SO_4 , and filtered. After evaporation under reduced pressure, this phase yielded the ester derivatives.

Methyl caffeate (1): Yield 87.77%; IR ν_{max} (KBr, cm^{-1}): 3477, 3009, 2953, 1678, 1606, 1436. ^1H NMR (DMSO- d_6 , 200 MHz): δ_{H} 3.64 (3H; s), 6.24 (1H; d; $J=15.9$ Hz), 6.73 (1H; d; $J=8.1$ Hz), 6.96 (1H; dd; $J=8.1, 1.9$ Hz), 7.02 (1H; d; $J=1.9$ Hz), 7.45 (1H; d; $J=15.9$ Hz); ^{13}C NMR (DMSO- d_6 , 50 MHz): δ_{C} 51.7, 114.1, 115.1, 116.2, 121.9, 125.9, 145.6, 145.9, 148.9, 167.5.

Ethyl caffeate (2): Yield 86.53%; IR ν_{max} (KBr, cm^{-1}): 3435, 3180, 2981, 1658, 1452. ^1H NMR (DMSO- d_6 , 200 MHz): δ_{H} 1.19 (3H; t; $J=7.1$ Hz), 4.11 (2H; q; $J=7.1\text{Hz}$), 6.22 (1H; d; $J=15.8\text{Hz}$), 6.72 (1H; d; $J=8.2$ Hz), 6.96 (1H; dd; $J=8.2, 2.0$ Hz), 7.01 (1H; d; $J=2.0$ Hz), 7.43 (1H; d; $J=15.8$ Hz); ^{13}C NMR (DMSO- d_6 , 50 MHz): δ_{C} 14.6, 60.0, 114.3, 115.1, 116.0, 121.7, 125.8, 145.3, 145.9, 148.7, 166.9.

Propyl caffeate (3): Yield 85.96%; IR ν_{max} (KBr, cm^{-1}): 3460, 3099, 2968, 1666, 1442. ^1H NMR (DMSO- d_6 , 200 MHz): δ_{H} 0.89 (3H; t; $J=7.4$ Hz), 1.61 (2H; m), 4.04 (2H; t; $J=6.7\text{Hz}$), 6.24 (1H; d; $J=15.9\text{Hz}$), 6.73 (1H; d; $J=8.2\text{Hz}$), 6.98 (1H; dd; $J=8.2, 1.9$ Hz), 7.03 (1H; d; $J=1.9$ Hz), 7.45 (1H; d; $J=15.9$ Hz); ^{13}C NMR (DMSO- d_6 , 50 MHz): δ_{C} 10.8, 22.1, 65.6, 114.4, 115.2, 116.1, 121.8, 125.9, 145.4, 146.0, 148.8, 167.1.

Isopropyl caffeate (4): Yield 93.06%; IR ν_{\max} (KBr, cm^{-1}): 3466, 3311, 2983, 1680, 1371. ^1H NMR (DMSO- d_6 , 200 MHz): δ_{H} 1.22 (6H; d; $J=6.2$ Hz), 4.97(1H; m), 6.18 (1H; d; $J=15.9$ Hz), 6.73 (1H; d; $J=8.2$ Hz), 6.89 (1H; dd; $J=8.2, 2.0$ Hz), 6.99 (1H; d; $J=2.0$ Hz), 7.47 (1H; d; $J=15.9$ Hz), ^{13}C NMR (CD $_3$ OD, 50 MHz): δ_{C} 22.1, 68.9, 115.0, 115.6, 116.5, 122.7, 127.6, 146.3, 146.7, 149.5, 168.9.

Butyl caffeate (5): Yield 91.53%; IR ν_{\max} (KBr, cm^{-1}): 3412, 2960, 1722, 1442, 1163. ^1H NMR (DMSO- d_6 , 500 MHz): δ_{H} 0.88(3H; t; $J=7.4$ Hz), 1.39-1.31(2H; m), 1.63-1.54(2H; m), 4.09 (2H; t; $J=6.6$ Hz), 6.24 (1H; d; $J=15.9$ Hz), 6.74 (1H; d; $J=8.3$ Hz), 6.97 (1H; dd; $J=8.3, 2.1$ Hz), 7.03 (1H; d; $J=2.1$ Hz), 7.44 (1H; d; $J=15.9$ Hz); ^{13}C NMR (DMSO- d_6 , 125 MHz): δ_{C} 14.0, 19.1, 30.8, 63.9, 114.5, 115.2, 116.2, 121.8, 125.9, 145.4, 146.0, 148.8, 167.1.

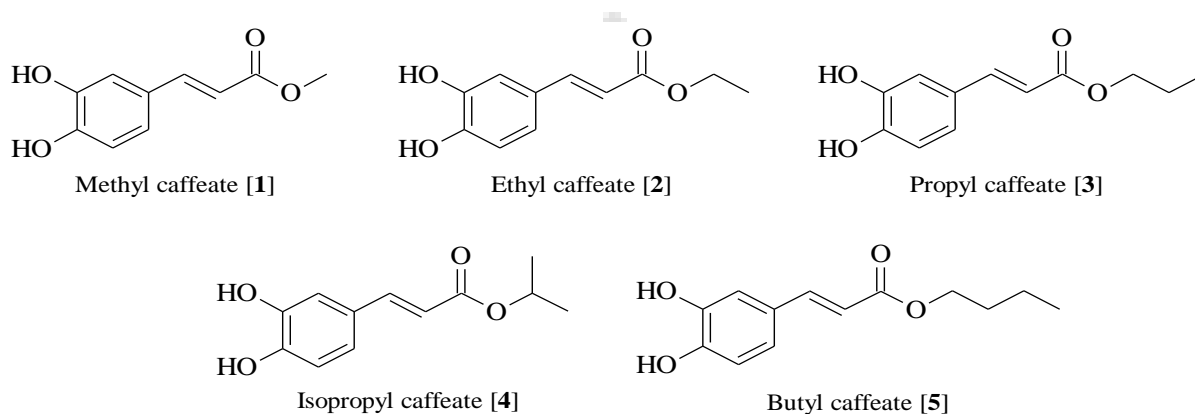


Figure 1. Chemical structures of the evaluated compounds.

Antifungal assay

Microorganisms in microbiological test were used strains of *Candida albicans* (ATCC-76645, LM-120 and LM-106), two strains of *C. krusei* (LM-08 and LM-13) and three strains of *C. tropicalis* (ATCC-13803, M-14 and M-7A). The strains were respectively acquired from the Adolfo Lutz Institute in São Paulo, and from the Federal University Pharmaceutical Science Mycology Laboratories of São Paulo and Paraíba. The yeast strains were maintained in appropriate medium, Sabouraud Dextrose Broth-SDB (Difco Laboratories, USA, France), and stored at 4°C and 35°C. The microorganism suspension was prepared according to McFarland tube 0.5, and adjusted by means of a spectrophotometer (Leitz-Photometer 340-800) at 90% T (530 nm) corresponding to approximately 10^6 CFU mL^{-1} [13].

Culture Medium: The antifungal activity assays were performed in Sabouraud Dextrose Broth-SDB (Difco Laboratories, France, USA), which was prepared and used according to manufacturer's instructions.

Determination of the minimum inhibitory concentration (MIC): The MIC value was determined by microdilution method using 96 well "U" shaped microtiter plates in duplicate. In each well of the plate 100 µl of twice concentrated SDB liquid medium was added. Then, 100 µL of product solution (also doubly concentrated) was placed in the first row of plate wells. Through serial dilution (ratio of two), the concentrations of 1024 µg/mL to 32µg/ml were obtained, such that in the first line of the plate was the highest concentration and in the latter, the lower concentrations. Finally, 10 µl of inoculum was added to the wells in each plate column that specifically referred to a strain. The same was also done in the culture medium with the fungal drug nystatin (100 IU). The plates were incubated at 37 °C for 24-48 h. For each strain, the MIC was defined as the lowest concentration capable of inhibiting fungal growth in the wells as visually observed compared with the control. All tests were performed in duplicate and the results were expressed as a geometric mean of the MIC values obtained in both tests [14].

RESULTS

The results obtained in this study revealed that all the studied compounds, with the exception of butyl caffeate (**5**), presented weak to moderate antifungal activity. Among the five evaluated ester derivatives, the methyl caffeate (**1**) was found to exhibit the best antifungal activity, with MIC values of 512µg/ml against all strains of *C. albicans*, *C. tropicalis* and *C. krusei* screened. Compounds ethyl caffeate (**2**), propyl caffeate (**3**) and isopropyl caffeate (**4**) presented the same activity on all *Candida* species, with MIC=1024 µg/ml and butyl caffeate (**5**) did not show antifungal effect. Further, all strains tested showed precisely the same degree of sensitivity to studied compounds. **Table 1** summarizes the *in vitro* susceptibilities of the eight *Candida* strains against all the test compounds.

Table 1. Minimal inhibitory concentrations (MICs) values (expressed in µg/ml) of the compounds 1-5.

Compounds	MIC (µg/mL)							
	<i>C. albicans</i> (ATCC-76645)	<i>C. albicans</i> (LM-120)	<i>C. albicans</i> (LM-106)	<i>C. tropicalis</i> (ATCC-13803)	<i>C. tropicalis</i> (M-14)	<i>C. tropicalis</i> (M-7A)	<i>C. krusei</i> (LM-08)	<i>C. krusei</i> (LM-13)
1	512	512	512	512	512	512	512	512
2	1024	1024	1024	1024	1024	1024	1024	1024
3	1024	1024	1024	1024	1024	1024	1024	1024
4	1024	1024	1024	1024	1024	1024	1024	1024
5	+	+	+	+	+	+	+	+
Micro-organism	+	+	+	+	+	+	+	+
Nystatin	-	-	-	-	-	+	+	-

+: Growth of micro-organism -: No growth of micro-organism

Some structural features of the screened ester derivatives that may alter the antifungal activity (alkyl ester side chain length) were observed, giving information on possibly functional groups important for the antifungal effect.

A close examination of the structures of the studied compounds permitted to conclude that the introduction of bulky alkyl substituents on the ester side chain resulted in decreased of the antifungal effect. For example, compound methyl caffeate (**1**; methyl group) was found to be the most bioactive compound (equal MIC = 512 µg/ml), whereas the compound butyl caffeate (**5**; butyl group) was inactive. This reduction in the antifungal activity probably occurs due to steric hindrance caused by the presence of bulky butyl group and/or increasing the lipophilicity.

DISCUSSION

In the current work, a set of five caffeic acid ester derivatives (methyl caffeate, ethyl caffeate, propyl caffeate, isopropyl caffeate and butyl caffeate) with different substitutions on the ester side chain was evaluated for its *in vitro* antifungal activity against several *Candida* strains,

namely *C. albicans* ATCC-76645, *C. albicans* LM-120, *C. albicans* LM-106, *C. tropicalis* ATCC-13803, *C. tropicalis* M-14, *C. tropicalis* M-7A, *C. krusei* LM-08 and *C. krusei* LM-13. The antifungal assay was carried out in 96-well microplates (microdilution method) and the results were expressed as minimal inhibitory concentrations (MICs) in µg/ml.

Earlier investigations have documented the antifungal activity of cinnamic acid derivatives, showing the relevance of this paper [12-16]. For example, Narasimhan and collaborators [11] investigated the antifungal activity of a series of nine esters of cinnamic acid (methyl cinnamate, ethyl cinnamate, propyl cinnamate, isopropyl cinnamate, butyl cinnamate, isobutyl cinnamate, octyl cinnamate, phenyl cinnamate and benzyl cinnamate) against two fungi: *Candida albicans* and *Aspergillus niger*. All the screened esters showed potent antifungal activity against both *A. niger* and *C. albicans*, principally the compound isobutyl cinnamate, with MIC values of 12.0 and 14.0 µM respectively. The MIC values of the other cinnamic acid ester derivatives varied between 36.0 and 61.0 µM for the filamentous fungus *A. niger*, and between 43.0 and 61.0 µM for the yeast *C. albicans*.

Moreover, most of the compounds evaluated in this study was earlier scrutinized on different biological activities, such as cytotoxic [17,18], anti-inflammatory [19,20], antioxidant [21,22], leishmanicidal, trypanocidal [23], antibacterial [11,24] and antihypertensive [25] activities.

In general, it could be clearly recognized that potential antifungal activity as well as fungicidal nature of the compounds is dependent on their chemical structures. Our findings suggest that the structure of the synthesized compounds herein needs to be further optimized to provide more insight into the structure-activity relationship of this class of compounds [26].

CONCLUSION

The present study investigated the antifungal activity of five caffeic acid ester derivatives against selected eight yeast strains belonging to the genera *Candida*. Further, through a preliminary Structure-Activity Relationship (SAR) study, we found that alkyl ester side chain length influences the antifungal activity in this series. Future research adding new derivatives to this collection may be interesting in the search for new antifungal compounds.

CONFLICT OF INTERESTS

Author declares that he has no conflict of interest.

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