

Evaluation of the antifungal activity and modulation between *Cajanus cajan* (L.) Millsp. leaves and roots ethanolic extracts and conventional antifungals

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ABSTRACT

Background: The use and investigation of natural products with antimicrobial activity from vegetal source have been reported by several researchers. *Cajanus cajan* (Fabaceae) is a multiple use specie mainly as human food. In popular medicine, diverse parts of the plant are used as sedative and to treat cough, hepatitis, and diabetes. **Materials and Methods:** This study shows the characterization of secondary metabolites present in ethanolic extracts from leaves and roots of *Cajanus cajan* by phytochemical prospection. The evaluation of the antifungal activity was performed by the microdilution method, and from the subinhibitory concentrations (MIC 1/8) the modulatory activity of antifungal (fluconazole and ketoconazole) was analyzed by the direct contact assay against *C. albicans* ATCC40006, *Candida krusei* ATCC 6538 and *Candida tropicalis* ATCC 40042. **Results:** The results showed the presence of tannins, flavonoids, and alkaloids in both extracts as the clinically relevant antifungal activity. The modulatory potential is presented by the antifungal tested against yeasts. **Conclusion:** The extracts studied here have demonstrated to be a new therapeutic source to treat these microorganism-associated diseases.

Key words: Antifungal activity, *Cajanus cajan*, phytochemical prospection, modulatory activity

INTRODUCTION

The increasing incidence of adverse side effects associated with conventional drugs, and the emergence of resistance to antibiotics^[1,2] justifies the search for new drugs from vegetable species in order to combat the multiresistant microorganisms.^[3,4] The study of natural products from vegetable origin presenting antimicrobial activity has been shown in many perspectives mainly because of the presence of tannins, alkaloids, saponins, and terpenes. This illustrates the importance of natural products as sources of new antimicrobials agents.^[5]

Cajanus cajan (L.) Millsp (Fabaceae) is a annual perennial shrub largely cultivated in tropical and subtropical regions of the world, typical to dry climates.^[6] Its seeds are sources

of vitamins, minerals, and proteins,^[7] and it is popularly known as gaundú, ervilha de pombo among others.^[8] Although there are few studies regarding the phytochemical profile of the roots in comparison with ones about *C. cajan* seeds, these different parts of the plant are used in traditional medicine as sedative and to treat cough, hepatitis, and diabetes.^[9,10]

This work is aimed to perform the phytochemical prospection and to evaluate the antifungal activity of ethanolic extracts from *C. cajan* fresh leaves and roots. Moreover, the possible modulator effect of fluconazole and ketoconazole was also analyzed.

MATERIALS AND METHODS

Plant material obtention

C. cajan leaves and roots were collected from Gavião farma, Missão Velha municipality (Ceatá States, Brazil) in April 2010. A voucher specimen was sent to the Herbarium Cariense Dárdano de Andrade Lima, which is deposited on the registration #5108.

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Preparation of extracts and phytochemical prospection

Fresh triturated leaves (700 g) and roots (862 g) were exhaustively extracted with cold ethanol for 72 h.^[11] After this, the solvent was distilled in a rota evaporator under reduced pressure to give 200 g (leaves extract) and 180 g (roots extract). Phytochemical tests to detect the presence of secondary metabolites were performed following the method described by Matos.^[12] These tests were based on the visual observation of color modification or precipitate formation after the addition of specific reagents.

Antifungal activity evaluation – minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC)

The minimal inhibitory concentration (MIC) was obtained by a broth microdilution assay according to NCCLS guidelines.^[13] Before the tests, fungi were activated in Sabouraud medium (48 h, 35±2°C). Three ATCC standard strains provided by Oswaldo Cruz Foundation – FIOCRUZ were used: *Candida albicans* ATCC40006, *Candida krusei* ATCC 6538, and *Candida tropicalis* ATCC 40042.

After the subculture, Each culture was mixed with a sufficient volume of sterile supplemented Sabouraud broth to achieve a turbidity equivalent to that of McFarland's no. 0.5 standard. To obtain the test inoculum, this suspension was further diluted with the same culture medium to approximately 1×10^6 colony-forming units (CFU)/mL immediately before use. Each dilution measuring 100 µL were distributed in 96-well plates plus extracts in different concentrations, achieving 5×10^5 UFC/mL as the final concentration of the inoculum.

Extracts and fractions were dissolved in distilled water and dimethyl sulfoxide (DMSO) to concentration of 1024 µg/mL. Further serial dilutions were performed by addition of the broth to reach a final concentration in the range of 512 a 8 µg/mL. All experiments were performed in triplicate, and the microdilution trays were incubated at 35±2°C for 24 h.

The plates containing the fungal strains were analyzed and the turbidity of the broth as the indication of growth of microorganisms.^[14] MIC was defined as the minimal concentration able to inhibit the yeast growth in comparison to positive control. The fungal culture that did not present growth was used to inoculate plates containing solid medium in dextrose potato agar (PDA) in order to obtain the minimal fungicidal concentration (MFC).^[15,16]

Fluconazole and ketoconazole modifying activity

The evaluation of ethanolic extracts as antifungal activity modifiers was performed using fluconazole and ketoconazole MICs obtained in the presence and absence

of the microdilution method modified according to CLSI/NCCLS M7-A6 guideline.^[13] Inhibitory concentration (MIC 1/8, 10% Sabouraud broth) were used against all strains previously described. Fungi were exposed to extracts that presented MICs ≤ 512 µg/mL.

The antifungal solutions (1024 µg/mL) were prepared in distilled water for use on the same day. A total of 100 µL of the antifungal solution, using serial dilutions (1 : 2), was added to the wells containing 10% sabouraud and the diluted fungal suspension (1 : 10). Final antifungal concentrations were 512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 µg/mL. Microplates were incubated for 24 h at room temperature and the modulatory activity was determined by visualization of turbidity.

RESULTS AND DISCUSSION

Phytochemical prospection

Phytochemical prospection of *C. cajan* leaves and roots ethanolic extract indicated the presence of tannins, flavonoids and alkaloids. These results are in concordance with other studies performed with *C. cajan* leaves extracts too, where these same secondary metabolites classes were found.^[17,18] Other reports quantified and isolated flavonoids in leaves extract (quercetin, luteolin, apigenin, and isorhamnetin)^[19,20] and roots of this specie.^[21,10]

Biological activities are described for different secondary metabolites classis. Although the antifungal propriety of flavonoids is well known, flavonoids also possess antibacterial, antilipoperoxidative and antiparasitic activities.^[22,23] Tannins are reported to exhibit bactericidal and fungicidal properties^[24] and alkaloids are antitumor, antiinflammatory and antimicrobial.^[25]

Evaluation of antifungal activity (MIC) and minimal fungicidal concentration (MFC)

As shown in Table 1, the ethanolic extracts obtained from *C. cajan* inhibited the growth of yeasts strains. The leaves extracts presented a MIC of 256 µg/mL against *C. albicans* and *C. tropicalis*.

Leaves and seeds from *C. cajan* have been extensively studied because of the ethnopharmacological properties. Diverse activities have been elucidated as the anticandidal. In the study performed by Runyoro *et al.*^[26] using the methanolic extract of leaves, a moderate activity against *C. albicans* was verified by the bioautography method. However, according to Bolle *et al.*^[27] this method may be not accurate because it can induce the compound decomposition.

Ethanolic extracts from *C. cajan* leaves were very active against *C. albicans*^[28] using the cavity and disk diffusion

Table 1: Minimal inhibitory concentration of *C. cajan* leaves and roots extracts

Extract	Strains MIC (µg/mL)		
	<i>Candida albicans</i>	<i>Candida krusei</i>	<i>Candida tropicalis</i>
EEOHFL	256	512	256
EEOHFR	512	512	512

EEOHFL: Ethanolic extract of fresh leaves, EEOHFR: Ethanolic extract of fresh roots

methods. This result corroborates our findings that can be considered clinically relevant^[29] as the extract presented MIC ≤ 1000 µg/mL against all strains tested.

When Braga et al.^[17] tested methanolic extracts of leaves and seeds were tested against *C. albicans* e *Cryptococcus neoformans*, the inhibition halos were considered to be very active, but a MIC value of 2500 µg/mL was obtained against *C. albicans*. A value much higher than that one found here. This can be explained by extracts of this species in different regions of Brazil can lead to extracts with different chemical composition from that previously reported.

The extracts studied here presented MFC ≥ 1024 µg/mL against all *Candida* species suggesting that the extracts can be fungistatic too.

Evaluation of fluoconazole and ketoconazole modifying activity

The modifier effect of fluoconazole and ketoconazole activity are shown in Table 2. It is possible to see a strong synergism between both antifungal and *C. cajan* extracts (MIC 1/8) against *Candida* species.

According to studies performed by Giordani et al,^[30] essential oil from *Thymus vulgaris* and *Origanum vulgare* were strongly active against *Candida albicans*. Besides this, the essential oil from *T. vulgaris* potentialized the action of amphotericin B leading to the complete inhibition of fungal growth.

Table 2 shows that both antifungal had their activity potentialized by leaves extracts against *C. albicans*. Roots extract, in combination with the fluoconazole and ketoconazole, presented better effects against the other *Candida* species. This is the first report of a natural product potentializing the fluoconazole and ketoconazole effects.

CONCLUSION

The phytochemical prospection of *C. cajan* leaves and roots extracts have demonstrated the presence of diverse class of compound and these compounds are related to known

Table 2: Minimal inhibitory concentration of antifungal alone or associated to *C. cajan* extracts against *Candida* species

	Modulatory activity (µg/mL)					
	<i>Candida albicans</i>		<i>Candida krusei</i>		<i>Candida tropicalis</i>	
	F	K	F	K	F	K
ExtEtOHFF	32	≤0,5	64	16	16	≤0,5
ExtEtOHFR	512	16	64	8	4	≤0,5
C+	512	128	64	16	32	≤0,5

F: Fluoconazole; K: Ketoconazole; C+: antifungal; EEOHFL: ethanolic extract of fresh leaves; EEOHFR: ethanolic extract of fresh roots

biological activities (as antifungal). The leaves extract presented best activity against *C. albicans* and *C. tropicalis*. However, the evaluation of antifungal modifying activity suggest that the extracts studied here demonstrated to be a new therapeutic source to treat diseases associated to the yeasts resistant to conventional antifungal. The results are promising and they can stimulate future researches regarding the phytochemical, toxicological, and pharmacological aspects of natural products from *Cajanus cajan*.

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