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Research article

# In-vitro antifungal study of various solvent extracts of costus specious (j. Koenig) sm. and costus pictus d. don against candida species

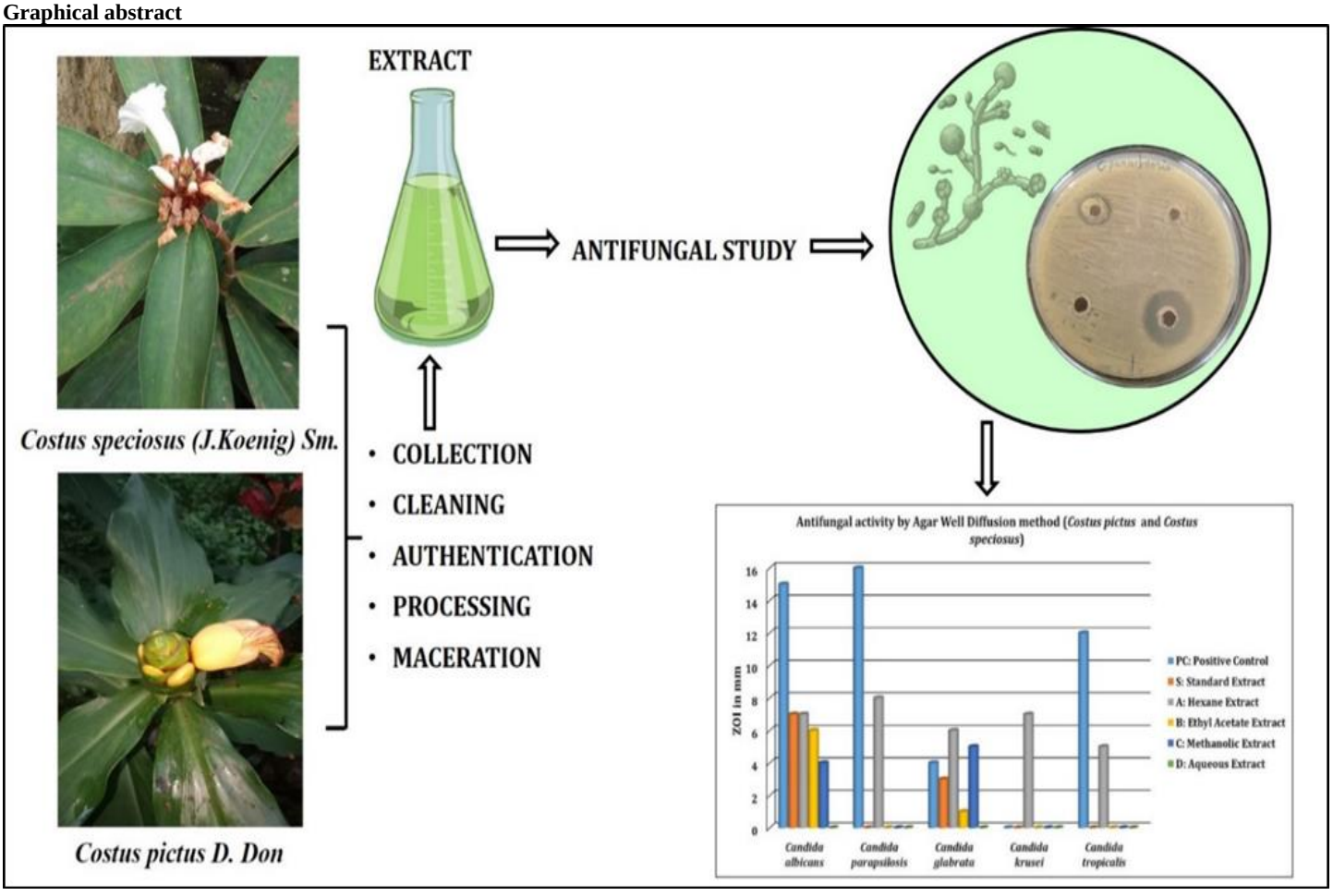
Shaikh Sana S*, Abubakar SB, Barrawaz Aateka Y, Dehghan MH, Mohammad Abdul J

Y.B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Aurangabad, Maharashtra, India

## ABSTRACT

The present investigation includes evaluation of various solvent extracts of Costus specious (J. Koenig) Sm. and Costus pictus D. Don. for in-vitro antifungal activity against selected Candida strains. The selected plants belong to the family Costaceae. Solvents such as hexane, methanol, water, and ethyl acetate of Costus pictus and standard extract of Costus specious were evaluated against Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis, and Candida krusei. The susceptibility of the fungal strains against the extracts was assessed by the agar well diffusion method. Clotrimazole was used as a positive control. The zone of inhibition was determined for each extract in comparison with the positive control. Results were recorded after 72 h. Of the various extracts analyzed, the hexane extract exhibited a potential antifungal activity overall. The methanolic, ethyl acetate extract of Costus pictus and standard extract of Costus specious shows some degree of activity against C. glabrata and C. parapsilosis. The aqueous extract shows no antifungal activity against the selected fungal strains. The above study indicates that C. krusei was more resistant to the solvent extracts; however, C. albicans and C. glabrata were more susceptible. Hence, Costus specious (J. Koenig) Sm. and Costus pictus D. Don demonstrate promising antifungal potential.

**Graphical abstract**



**Keywords:** Antifungal, Costaceae, Candida, Costus specious, Costus pictus.

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**Correspondence:** Sana Saffiruddin Shaikh* ✉ sana.shaikh310194@gmail.com

Y.B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Aurangabad, Maharashtra, India.

## INTRODUCTION

Over the past 25 years, the dominance of fungal infections has increased remarkably and consistently worldwide. [1]. Oral candidiasis is the most active and recurrent fungal infection [2] caused by filamentous fungi and yeasts, most notably Candida and Aspergillus. [3]. The most common species responsible for oral candidiasis are C. albicans, C. tropicalis, C. glabrata, C. dubliniensis, C. parapsilosis, C. orthopsilosis, C. metapsilosis, C. krusei, C. famata, C. guilliermondii, and C. lusitaniae. [4,5]. In general, amphotericin B based preparations and azole antifungal agents play a vital role in treatment [6]. The mainstream treatments available are becoming severe and unmanageable; therefore, there is an urge for alternative medicines [7]. Since the start of the human era, plants have been recognized as the origin of medicine and serve as potential with lesser side effects. [8]. The various alternative systems of medicine such as Ayurveda, Unani, Siddha, and Chinese traditional medicine are cited with evidence favoring treatment of different diseases.[9]. Many plants have been used as antifungal agents [10] against oral candidiasis [11,12].One such species, Costaceae belonging to the family of Zingiberaceae, propagates in India in the Himalayan region, Maharashtra, Karnataka, and Kerala. [13]. The Costus spp. commonly grown as medicinal and ornamental plants is also used as a dietary supplement to manage many diseases worldwide. [14]. The C. specious [CS] is also known as crepe ginger. [15,16]. The name C. specious was changed very recently to Hellenia speciosa (J.Koenig ex Smith) S. Dutta [17,18].The pharmacological actions such as antioxidant, antibacterial analgesic, antidiabetic, anti-inflammatory, antidiuretic, antifungal reported for C. specious [19,20].C. pictus[CP], another ornamental plant belonging to the family of Costaceae, is also well known as fiery Costus, insulin plant, spiral flag, and step ladder [21,22].The Rhizome and leaves show antidiuretic, antibacterial, anti-anthelmintic, and antitumor activities. [23]. It also possesses hypoglycemic and anti-inflammatory action. [24,25]. Previous literature reported for CS and CP shows the presence of potential antifungal activity. [26,27]. Therefore, the main purpose of the current study was to perform the antifungal activity of various solvent extracts of C. specious and C. pictus on selected fungal strains responsible for causing oral candidiasis.

## MATERIAL AND METHOD

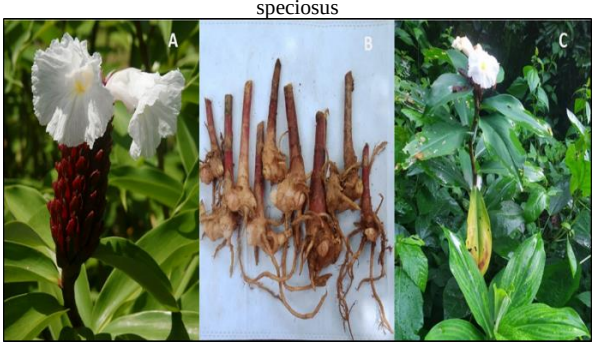
### Chemicals

Analytical grade chemicals such as hexane, ethyl acetate, methanol, was procured from Sigma Aldrich, Germany. Sabouraud dextrose agar was obtained from Hi-media laboratories, India.

### Collection, Identification, and Authentication of Plant Material

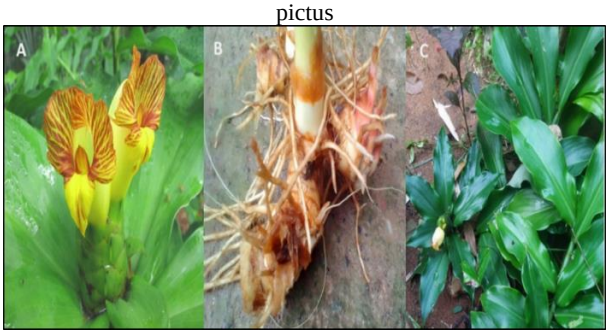
C. speciosus (Figure1) and C. pictus (Figure 2) healthy plants were collected from Usha nursery, Mallapuram district, Kerala.

Figure 1: C. speciosus (A) Flowers (B) Rhizomes (C) Complete plant of C. speciosus




Both the plants were deposited with vide accession numbers 722 and 723 in Herbarium, Department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India. The fresh leaves from C. pictus while the rhizome of C. speciosus was collected, washed thoroughly, and shade dried. [28,29]. The sample was powdered using a laboratory mixer grinder at high speed for 5 min and was stored in a tightly closed container for one day before being used for analysis.

Figure 2: C. pictus (A) Flowers (B) Roots (C) Complete plant of C. pictus




### Preparation of the Plant Extract

Aqueous Extraction About 2 g of powdered material (leaves) was extracted with 50 mL of water by the maceration process. The mixture was filtered by Whatman filter paper. The filtered solution was reduced to one-fourth of its original volume by using a rota-evaporator (IKA RV 10 digital V4 litre) at 40˚C to a constant weight until the volume giving the concentration of 160mg/mL. The solution was autoclaved at 121˚C and 15 lb pressure and stored at 4˚C for further studies. [30].

### Solvent Extraction (Cold Maceration)

2g of dried, powdered material (leaves) was weighed

accurately. The powder was macerated separately with ethyl acetate, hexane, and methanol with occasional stirring for 48 h. [31]. The mixture was filtered and then reduced to one-fourth volume at 40˚C with rota-evaporator and stored for further studies. [32,33].

**Antifungal Activity Studies**

Micro-organisms. Reference fungal strains were procured from the Government Medical College and Hospital Aurangabad (MS.) India. The strains comprise of Candida albicans (ATCC 90028), Candida parapsilosis (ATCC 2209), Candida glabrata (ATCC 64677), Candida tropicalis (ATCC 750), and Candida krusei (ATCC 14243). The fungal isolates were kept at 4˚C on a Sabouraud broth. The sample was sub-cultured for 24 h in Sabouraud broth at 37˚Cbefore any susceptibility test. [34].

**Media Preparation**

About 65 g of Sabouraud dextrose agar was dissolved in distilled water (1000 mL). The mixture was autoclaved for 15 min at 121˚C. Further, it was allowed to cool at room temperature. After cooling (about 45˚C), it was transferred into petri dishes. Each petri dish was left for cooling for about 30–35 minutes until completely set. [35].

**Agar Well Diffusion Test**

Each fungal strain was swabbed on the sterile agar plate using a clean and a sterilized cotton swab. Wells of about 7 mm diameter were made into the agar, and 50 µl of sample solutions and standard solution were added to each well. The concentration of crude extract used for the agar well diffusion method was 20 µg/µl. Clotrimazole (10 µg/µl) was used as a positive control. Positive control, negative control (various solvents used), and sample solution (all crude extracts) were added to each well and allowed to diffuse for 30 min at RT. Plates were incubated for 24 h at 35 ± 0.5˚C. The inhibition zones (mm) (ZOI)were recorded to determine the measure of antifungal activity. [36,37].

## RESULT AND DISCUSSION

The preliminary screening of antifungal activity for various solvent extracts and the standard extract was studied. The previous literature demonstrates the presence of phytochemicals such as terpenoid, phenols, and alkaloids mainly responsible for exhibiting potential antifungal effects. [38,39]. The presence of these phytochemicals [40] was confirmed in various solvent extracts [41] of C. speciosus and C. pictus. [42,43]. Henceforth the evaluation of antifungal activity, various solvents such as hexane, methanol, and ethyl acetate were selected. Five pathogenic fungal strains, i.e. Candida albicans, Candida parapsilosis, Candida glabrata, Candida tropicalis, and Candida krusei, were selected and evaluated using the agar well diffusion method (Figure 3).

The standard extract of CS and hexane extract of CP show the highest inhibition (7 mm each) against *C. albicans* (Table 1). In contrast, the ethyl acetate and methanolic extract show comparatively low activity. A potential antifungal activity was exerted by hexane extract against *C. parapsilosis* with a ZOI of 8mm. However, the other extract shows comparatively no activity. The hexane and methanolic extract show inhibition zones of 6 and 5mm against *C. glabrata*. Best antifungal activity against *C. krusei* was exhibited by hexane extract, while positive control and other extract show no inhibitory action. The Hexane extract shows inhibitory action against *C. tropicalis.* At the same time, the other extract shows comparatively no activity. The aqueous extract shows no activity overall with the selected fungal strains. The graphical representation for inhibition zones achieved by the agar well diffusion method is shown in (Figure 4).

Figure 3: Zone of inhibition for Agar well diffusion (A1and A2) *Candida albicans* (B1 and B2) *Candida glabrata* (C1 and C2) *Candida krusei* (D1 and D2) *Candida parapsilosis* (E1 and E2) *Candida tropicalis* (A: Hexane extract, B: Ethyl acetate extract, C: Methanol extract D: Aqueous extract, S: Standard extract, and PC: Positive Control)

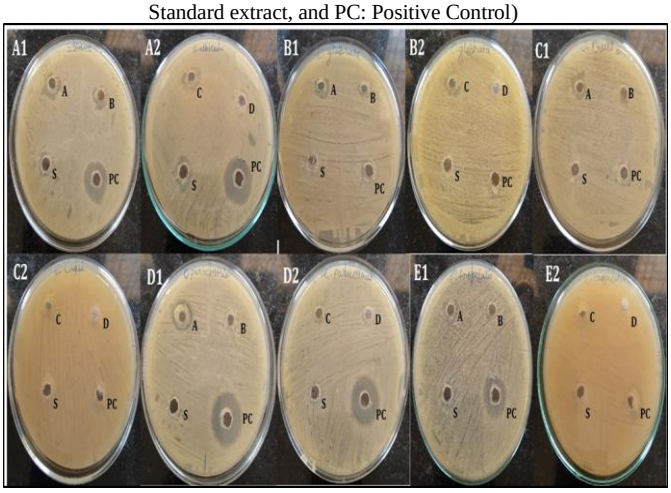
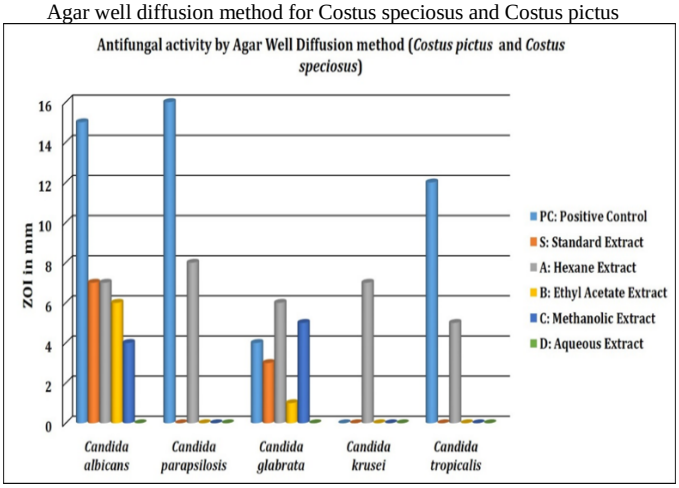



Table 1: Agar well diffusion method results. PC-Positive control, S-Standard extract. A-Hexane extract, B- Ethyl acetate extract, C- Methanolic extract, D-Aqueous extract.

| Micro-organism | Zone of inhibition ( in mm ) | | | | | |
|---|---|---|---|---|---|---|
| | PC | Costus speciosus | Costus pictus | | | |
| | | S | A | B | C | D |
| Candida albicans | 15 | 7 | 7 | 6 | 4 | - |
| Candida parapsilosis | 16 | - | 8 | - | - | - |
| Candida glabrata | 4 | 3 | 6 | 1 | 5 | - |
| Candida krusei | - | - | 7 | - | - | - |
| Candida tropicalis | 12 | - | 5 | - | - | - |

Figure 4: Graphical representation of the zone of inhibition achieved by Agar well diffusion method for Costus speciosus and Costus pictus



## CONCLUSION

The novelty of the present work is to evaluate antifungal activity for the plants *C. speciosus* and *C. pictus* against *Candida* species which was not reported earlier. The presence of these phytochemicals shows potential antifungal activity Henceforth the solvent extracts of both the plants were evaluated comparatively for the antifungal activity and ensure promising results as an antifungal agent. The average mean diameter for ZOI was recorded for various solvent extracts and positive control, which exhibited inhibitory action. The hexane extract shows potential inhibitory activity against the examined fungal strains. *C. krusei* was found to be more resistant as compared to other fungal strains. However, *C. albicans* and *C. glabrata* were found to be more susceptible to the hexane extract. The negative control used in the study does not show any remarkable activity against the selected strains. From the present study, the antifungal potential of both plants is validated. Henceforth these plants can be used as a potential antifungal agent. However, further work is needed for understanding the possible mechanism of action as an antifungal agent.

## CONFLICT OF INTEREST

All the authors have no conflict of interest

## ETHICAL APPROVAL

Not required

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