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Short Communication

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Anticandidal potential of *Crinum asiaticum* leaves extract against selected oral and vaginal *Candida* pathogens

Parveen Surain*, Kamal Rai Aneja

Department of Microbiology, Kurukshetra University, Kurukshetra, India

Abstract: Discovery of active principles in plants have given credence to the idea that integration of traditional medicine into the health care delivery would be very promising and should be encouraged. The incidence of *Candida* infections are rising at an alarming rate throughout the world. To overcome these alarming problem researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections. Different organic and aqueous extracts of *Crinum asiaticum* were screened for their anticandidal activity against five (9 isolates) human pathogenic *Candida* spp. by agar well diffusion assay. The pattern of growth inhibition varied with the six different solvents used for extraction. Out of six solvents, dichloromethane showed best activity against all tested *Candida* spp. with zone of inhibition ranging between 12.3mm and 20.6mm. Of the five *Candida* spp., *C.albicans* strain-2 was found to be most sensitive with maximum zone of inhibition of 20.6mm followed by *C.krusei* strain-1(17.3mm), *C.albicans* strain-4, *C.glabrata* (15.6mm) , *C.albicans* strain-1 (14.6), *C.albicans* strain-3 (12.3mm) and no activity was observed in *C.tropicalis, C.krusei* strain-2 and *C.lusitaniae*. The MIC tested for all the leaves extracts ranged between 100mg/ml and 6.25 mg/ml in dichloromethane extract. The result obtained in the present study suggests that the dichloromethane extract of *C.asiaticum* can be used in treating candidiasis caused by different species of *Candida*. however, further investigations will be needed for the isolation, characterization and exploitation of bioactive principles responsible for anticandidal activity.

Keywords: *Crinum asiaticum;* MIC; *Candida;* candidiasis; Inhibition; Characterization

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Candida spp. is the most common cause of fungal infections, leading to a range of life-threatening invasive to non-life-threatening mucocutaneous diseases. Under favourable conditions, it appears that over 20 *Candida* species can cause human infections. *C.albicans, C.glabrata, C.tropicalis* and *C.parapsilosis*, together account for about 95% of identifiable *Candida* infections The increasing numbers of immunocompromised patients, and the widespread use of certain medical and surgical practices, are favoring the emergence of normally commensal *Candida* species as life-threatening pathogens (Pfaller and Diekema, 2007; Achkar and Fries, 2010).

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions (Freydiere et al. 2003; Singh and Jain, 2011). To overcome these alarming problem researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections Discovery of active principles in plants have given credence to the idea that integration of traditional medicine into the health care delivery would be very promising and should therefore be encouraged. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Owoyale et al. 2005; Ubulom et al. 2011).

Among the plants investigated to date, one showing enormous potential is the *Amaryllidaceae* family, *Crinum asiaticum* commonly known as

Corresponding Author:
Surain P. (✉) Department of Microbiology, Kurukshetra University, Kurukshetra-136119, India
Email: dabas.ashi@gmail.com

Nagdovan in Gujarati, distributed throughout India. The name *Crinum* originates from the Greek "*Krinon*", which means "white lily", and they are commonly known as "Milk river, or veld lilies". It is an evergreen herbaceous plant of small to moderate size with greenish feathery leaves. The plant is used traditionally in warm infestation. Medicinally, it has been proven to possess various pharmacological activities like antitumour, anti inflammatory, antimicrobial, antiemetic, antiuretic, haemogogue, and anthelmintic activities. Further, studies reveal the presence of various phytochemical constituents mainly phenolics, alkaloids and fatty acids (Rahman et al. 2012; Refaat et al. 2012).

Traditionally, leaves and roots of this plant were used as emetic, diaphoretic and purgative. Leaves of *C. asiaticum* smeared with warmed castor oil are useful remedies for repelling inflammations and swellings at the end of toes and fingers. The plant is also used to treat inflammed joints and sprains. Slightly warmed juice of the leaves, mixed with a little salt, has been used for earache and other ear complaints. Roasted bulb is used as rubefacient in rheumatism. Bruised leaves of this plant act as an efficient insect repellent (Rahman et al. 2011). Therefore, the present study has been designed to assess the antimicrobial efficacy of leaves extracts of *C. asiaticum* against selected candidal pathogens.

MATERIAL AND METHODOLOGY

The leaves of *Crinum asiaticum* were collected from Ch. Devi lal park, Khizrabad. The taxonomic identity of this plant was confirmed by Dr. B.D. Vashishta, Chairperson of the Botany Department, Kurukshetra University, Kurukshetra

Extraction

The leaves samples were carefully washed under running tap water followed by sterile distilled water and air dried at 35-40°C for 4-5 days, homogenized to a fine powder using a sterilized mixer grinder and stored in air tight bottles. Six different solvents ethanol, methanol, acetone, petroleum ether, dichloromethane and sterile water were used for extraction. Ten grams of sample was separately soaked in conical flasks each containing 100ml of solvents (Akueshi et al. 2002; Ogundiya et al.2006). Each preparation was filtered through a sterilized Whatman No. 1 filter paper and finally concentrated to dryness under vacuum at 40°C using a rotaevaporator. The dried extracts, thus, obtained were sterilized by overnight UV-irradiation, checked for sterility on agar plates and stored at 4°C in labelled sterile bottles until further use (Aneja et al.2010).

Tested yeasts

A total of nine isolates belonging to five species of *Candida* were evaluated in this study. Of these, *C.albicans**-1(KC139703), *C.albicans**-2(KC139704), *C.krusei**-1(KC616317), *C.krusei**-2(KC616318), and *C.lusitanae**(KC610319) were identified on the basis of morphological, biochemical and molecular (28SrDNA sequencing) characteristics from the patients having vaginal and oral candidiasis, from the local clinics of Kurukshetra; and *C.albicans*-3 (MTCC No.3017), *C.albicans*-4 (MTCC No.4748), *C.glabrata* (MTCC No.3019), *C.tropicalis* (MTCC No.4690) were procured from Microbial Type Culture Collection, IMTECH, Chandigarh. These were subcultured on malt yeast agar (MEA) and incubated aerobically at 37°C. (*Nucleotide sequences of these yeasts have been submitted to GeneBank database).

Screening for anticandidal activity

Anticandidal activity of six solvent extracts (acetone, methanol, ethanol, petroleum ether, dichloromethane and aqueous) of the leaves was determined by the agar well diffusion method (Aneja et al. 2009). In this method, pure isolate of each yeast was subcultured on the MEA plates at 37°C for 24h. Minimum of four colonies of the isolates were transferred into normal saline (0.85%) under aseptic conditions. Density of each microbial suspension was adjusted equal to that of 10^6 cells/ml (standardized by 0.5McFarland standard) and used as the inoculum for performing agar well diffusion assay. 100µl of inoculum of each test organism was spread onto the MEA plates. The inoculated agar plates were allowed to dry, wells of 8mm were made with a sterile borer and the lower portion of each well was sealed with a little molten MEA medium. Each extract were reconstituted in 20% dimethylsulphoxide (DMSO) and 100µl of it was propelled directly into each well (in triplicates) of the inoculated agar plates for each test organism. The plates were allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at 37°C for 24h (Khokra et al. 2008). Sterile DMSO served as the negative control and ketoconazole as the positive control. The anticandidal activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone of inhibition was greater than 8mm (Aneja and Radhika, 2009). The experiments were performed in triplicates and the mean values of the diameter of inhibition zones with ± standard deviation were calculated.

Determination of minimum inhibitory concentration (MIC)

MIC is defined as the lowest concentration of a compound/extract/drug that completely inhibits the growth of the microorganism in 24h (Lee et al. 2004). The MIC of all the extracts was determined following the modified agar well diffusion method of Okeke et al. (2001). A twofold serial dilution of each extract was prepared by first reconstituting the powder in 20% DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 50mg/ml to 0.39mg/ml. A 100µl volume of each dilution was introduced into wells (triplicate) of the MEA plates already seeded with 100µl of standardized inoculum (10^6cells/ml) of the test microbial strain. All test plates were incubated aerobically at 37°C for 24 hrs and

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observed for the inhibition zones. The lowest concentration of the test extract showing a clear zone of inhibition (>8mm), considered as the MIC, was recorded for each test organism.

RESULTS

The anticandidal activity of *C. asiaticum* leaves extracts on the MEA plates varied in different solvents. Positive controls produced significantly sized inhibition zones against the tested *Candida* species, however, negative control produced no observable inhibitory effect against any of the test organism (Table 1).

A perusal of the data in Table 1 reveals that five of the six solvent extracts of *C.asiaticum* leaves tested possessed anticandidal activity against all the species of *Candida*. A literature search reveals that the leaves of *C.asiaticum* have received less attention on their anticandidal activity. The dichloromethane extract was found to be most effective against *C. albicans* strain-2 (20.6mm) followed by *C.krusei*-1 (17.3mm), *C.albicans* strain-4, *C.glabrata*, *C.albicans* strain-1 & 3. Among other organic extracts, petroleum ether extract was found to be best against *C. albicans* strain-2 with zone of inhibition of 18.6mm followed by other *C. albicans* strains. However, the aqueous extract displayed activity against three strains of *C. albicans* (1,2 &3), with zone of inhibition ranging between 12.3mm and 16.3mm. *C. albicans* strain-2 was found most sensitive yeast having an MIC of 25mg/ml. Other tested *Candida* species were found to be less sensitive, having MIC values ranging between 25mg/ml and 50mg/ml (Tables 1 and 2). Neem ras, ayurvedic syrup showed the inhibition against three strains of *C.albicans* (1,2,3) and *C. krusei* strain-1 with zone of inhibition ranging between 24.3mm and 11.3mm but the allopathic lotion containing ketaconazole, produced a zone of inhibition against all the tested species of *Candida* with zone of inhibition ranging between 40.6mm and 22.6mm (Tables 1).

DISCUSSION

Over the past few years, yeast belonging to the genus *Candida* continued to be among the most important

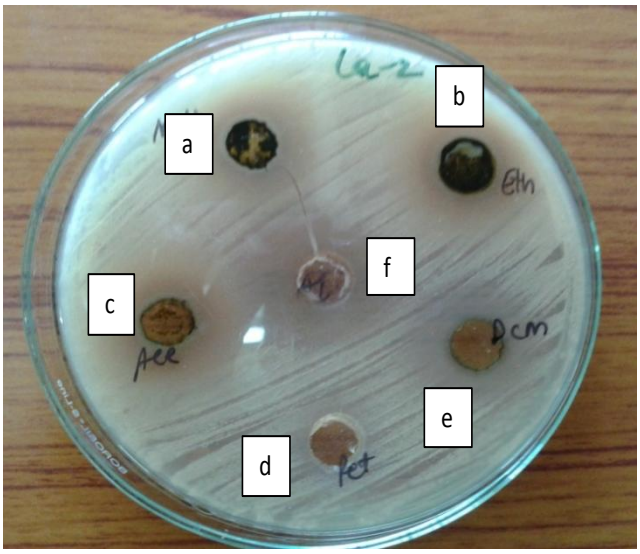

Figure 1 Zone of anticandidal inhibition against *C. albicans* strain-2 shown by six different extracts **(a)** methanol, **(b)** ethanol, **(c)** acetone, **(d)** petroleum ether, **(e)** dichloromethane, **(f)** aqueous extracts of *Crinum asiaticum* by agar well diffusion method

etiological agents of different human infections. The noticeable increase in the frequency of infections caused by non- *albicans Candida* species and the appearance of candidal isolates resistance to both Amphotericin B and the newer azoles represent two important alterations in the pattern of *Candida* infections. *C. asiaticum* have been used extensively against a wide range of ailments. The anticandidal potential of the leaves in six solvents (e.g. aqueous, petroleum ether, dichloromethane, methanol, ethanol and acetone) evaluated against species of *Candida*.

A majority of the described anticandidal effects of plant extracts have been attributed to their secondary metabolites (Das et al. 2012). The important sources of secondary metabolites described are flavonoids, lignans, aristolactams, terpenes, steroids, propenylphenols and alkaloids. It was found that the extract of *C. asiaticum* was possessed with some phytochemical metabolites especially the alkaloids such as crinamine and lycorine (Chen et al. 2011).

Table 1 Anticandidal activity of *C.asiaticum* leaves in six solvents against five species of *Candida* (9 strains) determined by agar well diffusion method on MEA medium

Solvent extracts	Diameter of zone of growth inhibition (mm)								
	Ca-1	Ca- 2	Ca-3	Ca-4	Cg	Ct	Ck-1	Ck-2	Cl
	C.asiaticum leaves extract								
Ethanol	16.3[a]±0.57	15.6±0.57 †	15.6±0.57	15.3±0.57	-	-	12.6±0.57	15.0±0.57	-
Methanol	14.6±0.57	17.6±0.57	17.3±0.57	14.6±0.57	-	-	13.6±0.57	-	-
Acetone	16.3±0.57	12.3±0.57	11.0±0.57	12.6±0.57	15.6±0.57	-	17.0±0.57	-	-
Petroleum ether	13.3±0.57	18.6±0.57	14.6±0.57	-	-	-	-	-	-
Dichloromethane	14.6±0.57	20.6±0.57	12.3±0.57	16.3±0.57	15.6±0.57	-	17.3±0.57	-	-
Aqueous	13.6±0.57	16.3±0.57	12.3±0.57	-	-	-	-	-	-
DMSO	0	0	0	0	0	0	0	0	0
Ketoconazole	40.6±0.57	41.3±0.57	31.3±0.57	26.0±0.57	30.6±0.57	25.3±0.57	35.3±0.57	22.6±0.57	33.6±0.57
Neem ras	16.3±0.57	15.6±0.57	17.6±0.57	-	-	-	15.6±0.57	-	-

Ca1, *C.albicans* strain-1; Ca2, *C.albicans* strain-2; Ca-3, *C.albicans* strain-3; Ca-4, *C.albicans* strain-4; Cg, *C.glabrata*; Ct, *C.tropicalis*; Ck1, *C. krusei* strain-1; Ck2, *C.krusei* strain-2; Cl, *C.lusitaniae*. **-** No activity; [a] Values, including diameter of the well (8mm), are means of three replicates

Table 2 MIC of *C.asiaticum leaves* in six solvents against five *Candida* species (9 strains) determined by modified agar well diffusion method on MEA medium

Solvent extracts	MIC (mg/ml)								
	Ca1	Ca2	Ca3	Ca4	Cg	Ct	Ck-1	Ck-2	Cl
C.asiaticum									
Ethanol	25	50	50	50	-	-	50	50	-
Methanol	50	25	25	50	-	-	50	-	-
Acetone	25	50	>50	50	50	-	25	-	-
Petroleum ether	50	25	50	-	-	-	-	-	-
Dichloromethane	50	12.5	50	25	50	-	25	-	-
Aqueous	25	50	25	-	-	-	-	-	-

Ca1, *C.albicans* strain-1; Ca2, *C.albicans* strain-2; Ca-3, *C.albicans* strain-3; Ca-4, *C.albicans* strain-4; Cg, *C.glabrata*; Ct, *C.tropicalis*; Ck1, *C. krusei* strain-1; Ck2, *C.krusei* strain-2; Cl, *C.lusitaniae*

The extensive survey of literature presents *Crinum* as an endless source of bioactive principles (Refaat et al. 2012). Within the huge number and diverse classes of phytoconstituents produced by this plant, members of this genus are best known as biofactories for *Amaryllidaceae* alkaloids. Phytochemical investigations led to isolation of several alkaloidal types and this current part of our review work summarized forty lycorine-type alkaloids isolated so far from *Crinums* as well as their structural differences and distribution in different *Crinum* species. Phytochemicals exert their antimicrobial activity through different mechanisms; tannins for example act by iron deprivation, hydrogen bounding or non-specific interactions with vital proteins such as enzymes (Rahman et al. 2011). The dichloromethane extract of *C. asiaticum* showed the highest anticandidal activity against all the tested strains of *Candida* of all the five species. Our results confirm the finding made by Swarnkar and Katewa (2009).

Conclusion

The findings of the present study authenticated the effective *in vitro* activity of *C. asiaticum* leaves extracts and it may be used to treat oral and vulvovaginal yeast species as they produce larger inhibition zones than the antifungal drugs often used to treat these pathogens. However, isolation of pure compounds and clinical investigation in animal models are to be made before their trials on humans.

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