SUSCEPTIBILITY OF CANDIDA ALBICANS CLINICAL ISOLATES TO SOME PLANT EXTRACTS IN SAUDI ARABIA

Khalil Abdullah, Mohamed Soliman and Mohamed Mohamed.
Department of Medical Lab Science, Faculty of Applied Medical Science King Khalid University, Saudi Arabia.

ABSTRACT

The aim of this study is to determine the occurrence of Candida albicans in clinical specimens and studying its susceptibility to some plant extracts in Saudi Arabia.

One hundred twenty eight human clinical specimens from 98 cases suffering from oral thrush and 30 cases suffering from vulvovaginitis were examined by mycological examination. C. albicans was identified by standard methods. The susceptibility of C. albicans to nine medicinal plants extract (alcoholic) marketed in Saudi Arabia was examined.

The frequency of C. albicans in the examined urine samples and oral swabs was 82.65 and 86.7%, respectively. Zone of inhibition (mm) of C. albicans against different plant extracts in different concentrations (500-32000 µg/ml) ranged from zero to 10±0.1. The minimum inhibitory concentration ranged from 4000 to 32000 µg/ml.

C. albicans is common in ICU cases either those with indwelling urinary catheter or those with oral thrush. The anti-candidal activity of the examined plants extracts (alcoholic) may help in treatment and prevention of candidiasis.

Key words: Candida albicans, clinical, plant extracts, antimycotics, and sensitivity

INTRODUCTION

Due to increased prevalence of drug resistant microorganisms there is great need to search for new effective drugs having natural or synthetic origin [1]. The resistance of pathogenic fungi, including C. albicans and non C. albicans species isolated from patient against anti fungal agents has increased [2]. Based on the toxicity and low potency, combined with the increasing side effects of these antimycotic drugs [3], novel fungal therapies with fewer side effects on humans are urgently required for effective management of candidiasis infections [4, 5]. Over the past decade, herbal medicine which has become a topic of augmented global importance has impacts on both world health and international trade. In terms of world health, traditional medicinal plants continue to play a center role in the health care system of large proportions of the world's population[6]. Recently, a number of researchers have reported the antimicrobial effects of various plant extracts against certain pathogens[4]. Medicinal plants have attracted considerable research attention as new sources of antimicrobial agents [7,8]. Wide varieties of plant extracts have antimicrobial and antimycotic effects and also anti-inflammatory properties [9,10]. There is a lack of available literature concerning the antimycotic effect of the marketed medicinal plants in Saudi Arabia. Candida species are members of the normal micro-biota with high prevalence in the normal population and can invade tissue and cause oral Candidiasis or life-threatening disease in patients whose immune defenses have been altered by old age, disease or iatrogenic intervention[11]. Possible risk factors causing an increase in Candida infections include prior antibiotic therapy, pregnancy, diabetes mellitus (DM), oral contraceptives containing estrogen and progestin, and immune-suppressed patients (transplanted patients, cancer patients treated with chemotherapy, and HIV patients)[12]. Vulvovaginal candidiasis (VVC) occurs at least once in 75% of women[13]. The aim of the present study was to assess the anti-candidal activities of nine plants extracts Acacia nilotica, Cardamom (Elettaria cardamomum), Datura stramonium, Dragon tree blood (Dracaena cinnabari Balf.f.), Ginger (Zingiber officinal), Neem (Azadirachta indica), Mangrove (Avicennia officinalis), Piperin (Piper nigrum) and Sedr (Zizphus spina-christi) against C. albicans isolated from human clinical cases.

MATERIAL AND METHODS

Clinical specimens:

The human clinical samples (No=128) including 98 urine samples and 30 oral thrush swabs were collected from ICU patients, King Faisal Hospital, Saudi Arabia during January-April, 2013. The examined cases age ranged from 40 to 70 years old including 98 female and 30 male. The specimens were transmitted within 4 hours to the laboratory of Microbiology, Faculty of Applied Medical Science, Bisha, King Khalid University for mycological examination. Each specimen was cultured on the surface of plates of Sabouraud dextrose agar (SDA) and incubated for 48 hours at 30° C. The isolated yeasts were identified by: Gram's stain, Colonial morphology on SDA, small, creamy, smooth, moist, raised, and the yeasty odor were considered the suspected colonies of Candida species. Gram stained films of suspected colonies were examined microscopically. They revealed Gram positive ovoid shaped budding cells. Subculture of suspected colonies of Candida on SDA slopes for 48 hours at 30° C followed by storage at 4 ° C to be used in further identification of colonies. All isolates proved to be Candida species by culture and microscopy were subjected to the following tests: Germ tube test, Chlamydospore production

and Sucrose assimilation[14]. The local ethics committee approved this study, and all subjects gave informed consent to the procedures. The statistical analysis was done using the chi-square test (p 0.05).

Plant Materials

Nine plant species were collected from Bisha market, Aseer, Saudi Arabia. The collected plants parts were air dried at room temperature, powdered and stored in dark colored bottles.

Preparation of crude extracts.

Two hundred and fifty grams of plant powder was soaked in 1.25-1.5 L of 95% ethanol for 5 days at room temperature, the mixture was mixed daily for regular infusion. After a five-day period, the extracted was filtered by using filter paper No1. The filtrate was dried by using a rotary evaporator at 60°C. The dried extract was stored in sterile glass bottles at -20°C until using[15]. The activity of the plant extracts was tested against C. albicans, inoculums containing 10^8 cells ml^{-1} were spread on the SDA. Antimicrobial activity test was then carried out by using the hole-plate diffusion method. Holes were made on the medium by using 6 mm cork borer. The dried plant extracts were dissolved in dimethylsulfoxide (DMSO) to final extract amounts of 500, 1000, 2000, 4000, 8000, 16000, 32000 µg⁄ml. Each hole (diameter 6 mm) in plate was filled with 50 µl of plant extract[16].

The inoculated plates were incubated at 37°C for 24 h. Zone to each hole was measured in millimeter, the inhibition zone is area surrounding the hole and there is no growth of inoculated microorganism. The lowest concentration of the plant extracts that didn't permit any visible growth of the incubated test organism in cultures was regarded as the MIC in each case[17].

All assays were carries out in triplicates to calculate the mean results.

RESULTS

Table (1): Occurrence of C. albicans in the examined clinical cases.

Source	No of cases	Positive		symptoms
		No.	Percentage	
Urine samples	98	81	82.65	Vulvovaginitis, fever, turbid urine or sterile pyuria
Oral swab	30	26	86.7	Oral thrush

Occurrence of C. albicans in the examined clinical samples was illustrated in Table 1. The obtained results showed that frequency of C. albicans from urine samples and oral swabs were 82.65% and 86.7%, respectively.

Table (2): Susceptibility of C. albicans to some plants extracts (alcohol) at different concentrations.

Plant name	Family	Plant used	Zone of inhibition (mm) ± SD at different concentrations of plant extracts						
			500 µg/ml	1000 µg/ml	2000 µg/ml	4000 µg/ml	8000 µg/ml	16000 µg/ml	32000 µg/ml
Acacia nilotica	Fabaceae	Leaves	0	0	0	0	0	5.5±0.1	8.5±0.1
Elettaria cardamomum	Zingiberaceae	Seeds	0	0	0	0	2.0±0.01	6±0.2	8±0.02
Datura stramonium	Solanaceae	Leaves	0	0	0	0	5±0.1	7±0.1	10±0.1
Dragon Blood tree	Convallariaceae	Resin	0	0	0	0	6±0.1	8±0.1	9±0.01
Ginger (Zingiber officinale)	Zingiberaceae	Rhizomes	0	0	0	0	0	7±0.2	10±0.1
Neem (Azadirachta indica)	Meliaceae	Leaves	0	0	0	5±0.1	7±0.1	8±0.1	10±0.1
Mangrove	Rhizophoraceae	Leaves	0	0	0	2±0.2	6±0.2	7±0.1	10±0.1
Black pepper	Piperaceae	Seed	0	0	0	2±0.2	5±0.2	8±0.1	10±0.1
Zizyphus (Sedr)	Rhamnaceae	Leaves	0	0	0	0	6±0.1	7±0.1	9±0.01

SD= Standard deviation

The plant extract that used in this study experiment were Acacia nilotica, Cardamom (Elettaria cardamomum), Datura stramonium, Dragon tree blood (Dracaena cinnabari Balf.f.), Ginger (Zingiber officinal), Neem (Azadirachta indica), Mangrove (Avicennia officinalis), Piperin (Piper nigrum) and Sedr (Zizphus spina-christi) against the reference strain of C. albicans. Data presente in Table 2 indicate that the alcoholic extracts of the tested plants extracts had inhibitoty effect against C. albicans. The inhibitory rate differe according to plant used. The zone of inhibition of the tested strains was zero for all plants extract at concentrations 500, 1000 and 2000 µg/ml. The zone of inhibition (range by mm) at concentration of 4000, 8000, 1600 and 3200 µg/ml were 0-5±0.1, 0-7±0.1, 5-8±0.1and 8-10±0.1, respectively. The obtained results indicated that Neem alcoholic extract had the most antifungal activities against the tested strains. The lowest antifungal action against C. albicans was Acacia nilotica.

DISCUSSION

Recently, the occurrence of C. albicans has greatly increased with the introduction of broad-spectrum antibiotics, immunosuppressive corticosteroids and prolonged antibacterial therapy. Among species of the genus Candida, C. albicans is the prevalent causative agent of candidiasis and constitutes the fourth most common nosocomial bloodstream isolate in industrial countrie[11]. In the present study (Table 1), the frequency of C. albicans in oral swabs were higher than that recorded previously (49.3%) by[18]**.** The variation in frequency of C. albicans may be due to stress. The high incidence of C. albicans among such human cases confirms the opportunistic nature of that organism which always taking advantages of stress factors or other disease conditions in the host[19]. The frequency of carriage of candidal colonization increased as a function of age[20]. A similar result was found previously by El-Sayed and Hamouda [21] who isolated C. albicans from vulvovaginitis cases with the percentage of 86.6%. Moreover, C. albicans was previously isolated from vaginitis cases with the frequencies of 67%, 84.9%, and 73% by Richter et al. [22], DeVos et al. [13] and Pirotta and Garland [23], respectively.

Plant extracts and their products are clinically safer than antibiotic[24]. Nowadays, the natural products and medicinal plants are subject of great global interest for the discovery of new antimicrobial agents[25].This could be related to the recent failure of antibiotics against the dramatic emerging of multidrug resistant pathogens in addition to the rapid spread of the new infections[26]. There is no available literature discussing the sensitivity of C. albicans against the tested plants extract in Saudi Arabia. Previous studies found that the ethanolic extracts of some Italian and Ethiopian plants have antimycotic activities against C. albicans. Further studies are needed to study the effect of combination of plants extracts as anti-candidal activities. [28,27]

The obtained results indicate that Neem (Azadirachta indica), Mangrove and Piper nigrum was very active against C. albicans. It inhibited the Candida growth at the four extract amounts (4000, 8000, 16000 and 32000 µg/ml. It had a minimum inhibitory concentration (MIC) of 4000 µg/ml and gave inhibition zone of 5.o±0.1, 2.0±0.2 and 2.0±0.2, respectively. Zizyphus (sedr), Dragon blood tree and Eletaria cardamomum also were active against C. albicans. It inhibited the Candida growth at three extract amounts (8000, 16000 and 32000 µg/ml. It had a minimum inhibitory concentration (MIC) of 8000µg/ml and gave mean inhibition zone of 6.o±0.1, 6.0±0.1 and 5.0±0.1, respectively. Ginger and Acacia nitolica seem less active against C. albicans. It inhibited the Candida growth at the two extract amounts (16000 and 32000 µg/ml). It had a minimum inhibitory concentration (MIC) of 16000 µg/ml and gave inhibition zone of 7.0.±0.2 and 5.5±0.1 respectively (Table 2).

Hassawi and Kharma [29] worked on C. albicans. It was resistant to the extract of Convolvulus althaeoides and it was affected by extract of Convolvulus arvensis at extract amounts (200 and 150mg/ml). This could be due to genetic variations between two species or higher concentrations of extract need to be used. Present results indicated that the tested plant extracts could have an anti-C. albicans effect. The active agent that caused the antimycotic activity of these medicinal plants against C. albicans need further studies to study the pharmacological action of it. Also, these plants extract may have the same agent but in different concentrations that causes high variations in their antimicrobial activity.

It could be concluded from the present study that candidiasis is a common nosocomial organism in ICU especially in those with indwelling urinary catheter and oral thrush in the current study. The tested plants extract having an antimycotic activity with various potencies and could be used for alternative medicine for treatment of candidiasis.

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حساسية العزلات الإكلينيكية للكانديدا المبيضة لبعض المستخلصات النباتية بالمملكة العربية السعودية

د/ خليل عبد الله خليل ، محمد طلعت عبده سليمان ، محمد السيد محمد محمد
قسم علوم المختبرات الطبية – كلية العلوم الطبية التطبيقية – جامعة الملك خالد – السعودية

تهدف الدراسة الحالية تحديد تواجد الكانديدا المبيضة في العينات الإكلينيكية لمرضى التهاب المهبل الشفري وسُلاَق الفم وكذلك دراسة حساسيتها لبعض المستخلصات النباتية بالسعودية.
لقد تم اجراء هذا البحث على مائة وثمانية وعشرون عينة إكلينيكية من 98 حالة تعانى من التهاب مهبلي شفري و30 حالة تعانى من سُلاَق الفم على التوالي. تم إجراء الفحص الفطري لها. وكذلك تم تعريف الكانديدا المبيضة بالطرق القياسية . تم عمل اختبار حساسية الكانديدا المبيضة لعدد 9 مستخلصات (كحولية) لنباتات مسوقة بالسعودية.
النتيجة: وجد إن معدل تواجد الكانديدا المبيضة في عينات البول ومسحات الفم 82.65% ، 86.7%على التوالي. وتراوح نطاق التثبيط (مم) من صفر إلى 10±0.1 وتراوح اقل تركيز تثبيطي من 4000 إلى 32000 ميكروجرام/ملى.
الاستنتاج: انتشرت الكانديدا المبيضة في حالات وحدة الرعاية المركزة محل الدراسة وخاصة المقسطرين وحالات سُلاَق الفم. وجد نشاط مضاد للفطريات للمستخلصات محل الدراسة. ويمكن ان تساهم تلك المستخلصات في العلاج والوقاية من العدوى بالكانديدا المبيضة.