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Invitro Antifungal Activity of Ziziphusmauritiana Extraction Against Clinical Isolates of Candida Spp at Khartoum State, 2021

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Abstract

Background: Medicinal plants have been discovered and used in traditional medicine and pharmaceutical industries since centuries. However, the antifungal activities of Z. mauritiana extracts on Candida Spps have not been discovered extensively.

Objectives: The present study aimed to investigate the antifungal activity of Z. mauritiana leaves methanol extracts against clinical isolate of CandidiaSpps.

Methods: an experimental biomedical study was carried out at Omdurman Maternal Hospital, Saudi Maternal Hospital and AlzaiemAlazhari University during the period from September to December 2021. 30 Candida species, of them 17 isolated from urine samples and 13 from vaginal swabs were included in this study. Antifungal activity was performed usingcup plate method using different concentrations of plant extraction (100%, 50% and 25%) and fluconazole(10µg). The statistical analysis was performed using SPSS version 20.

Results: The results showed significant increase in antifungal activity with increase in *Ziziphusmauritiana*concentration, while fluconazole showed best antifungal activity. However, the fungicidal concentration showed that Ziziphusmauritiana methanol extracts was able to inhibit Candia SPPs rather than C. albicans.

Conclusion: The finding of this study suggests that that Ziziphusmauritiana leaves extract showed promising results against Candida SPPs. Thus, it can be used as a source for functional ingredients for pharmaceutical drug industries in-order to reduce or inhibit fungal infection.

Keyword: Ziziphusmauritiana Concentration, Fluconazole, Candida SPPs

Introduction

Candidiasis is an infection caused by *Candida species*, which include: Oroesophageal candidiasis, paronychia, onychomycosis, perleche, respiratory infections, vulvovaginitis, thrush, pulmonary infection, eye infection, endocarditis, meningitis, fungemia or candidemia, or disseminated infection(1). The

incidence of life threating disseminated *Candida albicans*infections is increasing the hospitalized patient, with fatalities as high as 60%. Death from disseminated Candidiasis is significant percentage of cases is due to fungal invasion of the kidney, leading to renal failure. Treatment of Candidiasis is hampered by drug toxicity, the emergence of antifungal drug resistance and lack of vaccines against fungal pathogens[(2).] The polymorphic fungus *Candida species* is a member of the normal human microbiome. In most individuals, *Candida* resides as a lifelong, harmless commensal.Among them are molecules which mediate adhesion and invasion into host cells, the secretion ofhydrolases, the yeast-to-hypha transition, contact sensing andthigmotropism, biofilm formation, phenotypic switching and a range of fitness attributes .*Candida* is the most common etiological factor of opportunistic human fungal infections

Plants have been used in traditional herbal medicine for many years. In some parts of the world, plants and herbs are still the primary source of remedies used in treating diseases for instance, several plant extracts have been reported to have anti-Candida activitiesincluding *Ziziphusmauritiana*, *Alliumsativum* (Garlic) , Berberine-containing herbs , *Cinnamomumverum* (Cinnamon) and *Origanumvulgare* (Oregano) [(3)].

*Zizyphusmauritiana*has medicinal properties; it is used as antidote, diuretic, laxative andexpectorant. Its dried fruits are used as sedatives,anticancer, antipyretic, analgesic, appetizer, antihemorrhage agents and as the tonic[(3)]. Candidiasis is an infection caused by *Candida species*, Which include: Oroesophageal candidiasis, paronychia, onychomycosis, perleche, respiratory infections, vulvovaginitis, thrush, pulmonary infection, eye infection, endocarditis, meningitis, fungemia or candidemia, or disseminated infection[)4)] Here, in a preliminary investigation, we evaluated the in vitro activity Ziziphusmauritiana native to Republic of Sudan against healthcare-related pathogens with emphasis on C.albicans.[(5)]

Materials & Methods

– Study area and Population an experimental biomedical study was Carried out at Omdurman Maternal Hospital, Saudi Maternal Hospital and AlzaiemAlazhari University during the period from September to December 2021.The study includes pregnant women and Female in reproductive age with symptoms of vaginitis and/or puerperal sepsis. Any Patients known diagnosed with immunodeficiency or immunological disorders were excluded from Study.The sample size include30 Candida species, 17 isolated from urine samples and 13 from vaginal swabs.

– Ethical consideration

This study approved by ethical committee of AlzaiemAlazhari University, Saudi Material Hospital and Omdurman maternal Hospital.

Sampling processing

For urine samples: 5-10 ml of urine samples was centrifuged, discharge the supernatant urine, and then a dropped from the deposited urine was added to slide and covered it with cover glass, and then examined under microscope for round, oval or elongated yeasts with budding and with or without pseudohyphae.

For swab samples: Amies Transport Mediaprepared by adding 19.57 gram of Amies Transport powder to 1000 ml of distilled water was used for transportation.

– Samples Analysis

Gram stain:was done by staining with crystal to insure the microorganism is yeast.

Culture: Urine and swabs showed yeast cells in direct examination were cultured on plates ofsabouraud with streptomycin and penicillin and incubated at 35-37°C for 24-48 hours. The colony appeared small white or creamy with characteristic yeasty smell.

Germ tube test (GTT): A loop full of pure yeast growth was mixed with 0.5 ml of human serum in test tube, and then incubates the tube in water bath at 35-37°C for 2-3 hours. Transfer a drop from the preparation in to slide, cover it with cover glass and examine it microscopy using 40 xs.

Chromogenic media: A loop full from pure yeasty growth were inoculated on chromogenic media (*Himedia*™) which can differentiate *Candida species* based on specific chromogenic substances that hydrolysis enzymatically to give different colored product. *Candida albicans* produces green color colony, *Candidatropicalis* blue, *Candida krusei* fat pink and *Candida glabrata* produce smooth pink colony

Mueller Hinton agar: This medium was provided by (Himedia), it was prepared by adding 38 gram of Mueller Hinton powder to 1000 ml of distilled water and it was mixed well to be dissolved, then it was autoclaved for 15 minutes at 121^{o}c

MacFarland standard

1% v/v Sulfuric Acid: 1 ml of concentrated sulfuric acid was added to 99 ml of D.W. 1% w/v Barium chloride: 1 gram of barium chloride powder was added to 100 ml of D.W MacFarland Standard**:** 0.5 ml of 1% w/v of barium chloride were added to 99.5 ml of 1% sulfuric acid.

Sensitivity test:fresh leaves of the plant were collected, washed and cut into small sections,dried for three days and then ground to very fine paste/powder. The paste/powder was extracted aqoueously overnight followed by filtration. The organic solvent extract was put in tray of FreezDryer device at 60- °C till dryness.The residual extracts were dissolved in sterile water prior to antimicrobial testing .

Results

The higher significant inhibitory Effects was found with fluconazole(mean17.9 mm), followed by 100%,50% and 25% aqueous extract of *Ziziphusmauritana*Respectively (table 1). The best effect of fluconazole and plant extract was observed against *Candida* species rather than *Candida albicans*(table 2).

Table (1): Statistics and means differences of Fluconazole and 100%, 50% and 25% aqueous extract of *Ziziphusmauritana*

Antifunga l	Mean	Std. Deviation	Minimum	Maximum	P value	Multiple P values
(A) Fluconazole (n = 3 0)	1 7 . 9	2 . 5	1 3	2 1	**0 . 0 0 0**	A X B **0 . 0 0 7** A X C **0 . 0 0 0**

(B) 100% (n = 30)	16.0	2.7	11	19		X D **0.000** X C **0.010** X C **0.000** X D **0.002**
(C) 50% (n = 30)	14.1	2.7	10	19		
(D) 25% (n = 30)	11.8	3.01	7	17		

Table (2): Statistics and means differences of Fluconazole and 100%, 50% and 25% aqueous extract of *Ziziphusmauritiana* among isolated yeasts

Antifungal	***Candida albicans* (n = 20)**	***Candida species* (n = 10)**	**P value**
Fluconazole	17.60±2.798 (13-21)	18.70 ±2.003 (15-20)	0.278
Con 100%	15.75±2.954 (11-19)	16.50 ±2.461 (14-19)	0.496
Con 50%	13.60±2.604 (10-18)	15.10 ±2.961 (10-19)	0.166
Con 25%	11.20±2.858 (7-17)	13.10 ±3.071 (9-17)	0.105

Discussion

In this study out of 30 isolated *Candida, Candida albicans*20 was the most isolated species .This result is similar to study done in India by Roopa and Sunilkumar[6]who reported that *Candida albicans* was the most isolated species followed by *Candida tropicalis*, butt differ from done in India by Ramnder*et al*.,[7] who reported that *Candida krusei* was the most isolated species. This variation may be due to the number and type of specimens involved in each study.

In the present study 21 were sensitive to fluconazole, while 9 of total isolate were resistant.This result was disagreed with study done by Ahmed and Afnan[8]who showed that fluconazole was least effective against *Candida* species .This variation of results due to different concentration of antifungal used.

The present study also demonstrate antimicrobial effects of *Ziziphusmauritiana* extract which in the line with other researches confirming medicinal effects of this plant, done by Yan J and Cao J[9].

Conclusion

The results of the present study have clearly showed that Ziziphusmauritiana extract have acceptable effect against fungi, whileFluconazolewas the most effective one. Further studies with large numbers of samples and more advanced techniques are required to validate the results of the present study.

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