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In-vitro anti-candida activity of *ricinus communism* leaves extracts

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Abstract---The evaluation of in-vitro anti-Candida activity was done for *Ricinus communism* leaves extracts which are yielded by the extraction with different organic solvents (methanol, chloroform, and a combination of these solvents((1:1; v/v); methanol/chloroform)) against four *Candida* species(*tropicalis*, *kefyr*, *glabrata*, and *albicans*) by agar well diffusion method. In the present study, the results showed that all tested extracts possessed anti-Candida activity against all examined strains, but the leaves extracted by a combination of methanol and chloroform exhibited the best anti-Candida potentiality when compared with methanol or chloroform solvent which is separately used in the extraction process, in addition to that the anti-Candida activity of all leaves extracts was varied depending on the *Candida* species susceptibility and the concentration of the extract which used in every treatment, in addition to that the minimum inhibitory concentrations data of the combined solvents extract were (13, 18, 22, and 24% for *Candida glabrata*, *tropicalis*, *kefyr*, and *albicans* respectively.

Keywords---*Candida*, *Ricinus communism*, Anti-Candida.

Introduction

Infections induced by pathogenic microorganisms have become a major source of diseases and death cases in immunocompromised people in developing nations [1]. Despite the discovery of a significant number of antimicrobial drugs, pathogenic

microbes are constantly evolving resistance to them [2]. However, because many anti-mycotic therapies have unpleasant side effects or are extremely cytotoxic, cause re-emergence of the disease, cause drug-drug interactions, or could result in the emergence of resistance, some of them are ineffective [3] and hence less useful in therapeutic purposes. As a result, new antifungal drugs that are both more effective and less harmful are needed to address these drawbacks. Fungi, which include dermatophytes and *Candida* spp., are a crucial class of skin pathogens [4, 5] are significant. *C. albicans* and similar species can become pathogenic in some circumstances, usually linked with weakened patient immunity, producing oral, vaginal, and/or systemic candidiasis [6]. Vulvovaginal candidiasis (VVC) affects about 75% of adult women at some point in their lives, with *C. albicans* being present in 70–90% of cases [7]. *C. albicans* is known for targeting skin which can result in candidiasis occurrence, but it can also infect the esophagus and become systemic, resulting in a far more dangerous illness called candidemia [8, 9]. It also results in a broad range of diseases, from non-fatal mucosal candidiasis, such as vaginal yeast infections, thrush, skin rashes, and diaper rash, to lethal disseminated candidiasis in persons with weaker immune systems who have implants like pacemakers or artificial joints, or who use broad-spectrum antibiotics [10-12]. Toxicity of the currently available antifungal medications, *Candida* resistance to commonly used antifungals, recurrence of *Candida* infections, and non-cost effective antifungal drugs are some of the factors that limit the availability of effective antifungal medications. are all issues that *C. albicans* infections encounter [13]. Traditional medicine produced from plants is still used to relieve the problem of a limited supply of medications needed to treat candidiasis [14], finding new and effective anti-*C. albicans* compounds from plant sources were sparked by this. Synthetic medications, on the other hand, are reproduced using fundamental raw materials that are not renewable, such as petrochemicals and fossil fuels [15]. Plants continue to be a major source of novel lead compounds due to all of these advantages. Multiple drug resistance has emerged in human pathogenic bacteria as a result of the indiscriminate use of commercial antimicrobial medicines today [16]. Scientists were driven to look for novel and powerful antibacterial medicines to replace the current regimens as a result of this circumstance [17].

Ricinus communis L. (*R. communis*) is a soft woody little tree native to the tropics and temperate regions of the world [18]. It belongs to the Euphorbiaceae family. Several research using plant extracts as an antimicrobial activity for the release and development of novel antimicrobial compounds have been conducted [19-21]. In many nations, different plants are used medicinally as a source of powerful and effective drugs [22, 23]. The interest in this scientific study of *R. communis* leaves various indications of its efficacy in the treatment of a variety of disorders. The current study shows that methanolic, chloroform and a mixture of the two organic solvents extracts of *R. communis* L. leaves have anti-candida effects in vitro against *Candida* isolates.

The main aims of the present study are to investigate the anti-*Candida* activity of *Ricinus communism* leaves extracted by different organic solvents and evaluate the minimum inhibitory concentration of the potent extract against the studied pathogens.

Materials and Methods

Plant material

Fresh *Ricinus communism* leaves were collected from the home gardens of Kerbala in January 2021. To get the dust off the plants' leaves, tap water was used to wash them, then shade-dried at room temperature and ground into a fine powder by using an electrical grinder. To ensure uniform particle size, the powder was sifted through a sieve with a 2 mm hole size and kept at 4°C.

Preparation of leaves extracts

A 100 g of plant leaves was dissolved in 1L of methanol, chloroform, and a mixture composed of them (1:1 v/v). To avoid evaporation and sunlight exposure, All mixes were stored in sanitized beakers that were wrapped in aluminum foil for three days at room temperature. After three days, mixtures were filtered through Whatman No. 1 filter paper and maintained at 37°C in an incubator until all of the organic solvents had fully evaporated., after that, the produced extracts were dissolved in DMSO (Dimethyl sulfoxide) 20% to prepare the gradual concentrations (5, 10, 15, 20, and 25%) which needed for anti-*Candida* investigations [24,25].

Candida cultures

Only four species of *Candida* (*tropicalis*, *kefyr*, *glabrata* and *albicans*) were obtained from Postgraduate Laboratory in Biology department\ college of sciences\ university of Kerbala to screen the efficacy of the plant extracts against *Candida*. The yeast was grown overnight at 37° in Sabouraud Dextrose Agar scraped cell mass was diluted in 85 % NaCl solution, calibrated to McFarland 0.5 scale, and validated by spectrophotometric readings at 580 nm to create the inoculum for the assays. The cell suspension was then diluted to 10^4 UFCml⁻¹ for use in this study [26, 27].

Screening the anti-*Candida* activity of plant extracts

In a sterile Petri plate, newly prepared Sabouraud Dextrose Agar was coated with a *Candida* suspension equal to 0.5 McFarland Standard 10^4 UFCml⁻¹. Agar wells (which are created by using a 5 mm diameter sterile cork borer) were then filled with 0.1 ml of the extracts at a concentration of 5, 10, 15, 20, and 25 % and other wells were filled with 0.1ml of 100% 20 percent DMSO served as the experiment's negative control and clotrimazole as the positive control. The plates stand for about an hour to allow the extracts to properly diffuse into the medium. The plates were incubated at 37°C for 24 hours before being measured and checked for zones of inhibition. Each experiment was carried out three times [24].

The determination of Minimum Inhibitory Concentration

96-well microtiter plates were used for the serial dilution approach to determine the minimum inhibitory doses (MIC). The various plant extract concentrations were examined, and the very potent extract concentrations were serially diluted

with potato dextrose broth medium and the appropriate inoculum. The microplates were each incubated for 24 hours at 37 Co. MICs and were established as the lowest concentrations at which there was no discernible growth (under the binocular microscope) [28].

Statistical analysis

Analysis of variance (ANOVA) of the triplicate data was performed by using the data analysis tools in Microsoft Office 2010.

Results

The results of the present research showed that all the investigated crud extracts of *Ricinus communism* leaves (methanolic, chloroform, and the mixture of the two solvents) have anti-Candida potentiality against all tested yeast species, at the same time the efficacy of all extracts was vary depending on the solvent which used in the extraction process, the extracts concertation and the Candida species susceptibility as shown in table 1, 2 and 3.

The ability of methanol solvent in the extraction process was weak when compared with the chloroform solvent depending on the anti-Candida activity exhibited by the same gradual concentrations used from the two extracts (5,10,15, 20 and 25%) which were investigated against the same Candida species(*tropicalis*, *kefyr*, *glabrata*, and *albicans*), in the table (1) the highest anti-Candida potentiality was recorded with the highest concentration 25% against *Candida glabrata* and the inhibition zone was 57.33 ± 4.73 and in comparison with the positive control (Clotrimazole) which was 53.67 ± 3.79 , however, The data in the table (1) indicated that methanol extract inhibited the growth of other investigated Candida species weakly at low concentrations when compared with their positive controls.

Table 1: The anti-Candida activity of *Ricinus communism* methanol extract in concentrations (5, 10, 15, 20, and 25%) against *Candida* species (*tropicalis*, *kefyr*, *glabrata*, and *albicans*) respectively depending on inhibition zone diameters (mm)

Candida species	Methanol extract Concentrations					Clotrimazole	DMSO 20%
	5%	10%	15%	20%	25%		
<i>C. tropicalis</i>	9.67±1.53	17.00±2.65	29.00±1.00	37.67±2.89	51.00±5.29	51.67±0.58	00.00
<i>C. kefyr</i>	8.33±1.15	15.33±4.93	27.00±2.00	33.33±2.31	44.67±2.52	46.33±1.53	00.00
<i>C. glabrata</i>	12.33±1.53	19.33±2.52	31.67±1.53	42.33±2.08	57.33±4.73	53.67±3.79	00.00
<i>C. albicans</i>	5.67±0.58	9.33±0.58	16.33±2.08	25.00±2.00	34.33±4.16	33.00±2.00	00.00

The increase in growth inhibition of the studied Candida species can be observed by the data in table (2) that showed the high efficacy of chloroform in extraction which was very clear by the increasing of inhibition zones diameter with increasing in extract concentration which was the highest (80.00 ± 0.00) for each *Candida glabrata* and *Candida* triplicates at concentration 25%, these results represented the fungicidal action of the chloroform extract against the two Candida species.

Table 2: The anti-*Candida* activity of *Ricinus communism* chloroform extract in concentrations (5, 10, 15, 20 and 25%) against *Candida* species (*tropicalis*, *kefyr*, *glabrata*, and *albicans*) respectively depending on inhibition zone diameters (mm)

Candida species	Inhibition Zone Diameters (mm) (mean±SD)						
	chloroform extract concentrations					Clotrimazole	DMSO 20%
	5%	10%	15%	20%	25%		
<i>C. tropicalis</i>	16.00±2.65	27.33±3.06	42.00±4.36	57.00±2.65	80.00±0.00	51.67±0.58	00.00
<i>C. kefyr</i>	12.33±1.53	20.33±3.51	33.67±2.31	47.67±0.58	71.00±25.16	46.33±1.53	00.00
<i>C. glabrata</i>	21.00±1.00	32.67±2.08	46.67±0.58	59.00±2.65	80.00±0.00	53.67±3.79	00.00
<i>C. albicans</i>	9.00±1.00	18.67±0.58	24.00±1.00	37.33±1.53	44.33±2.52	33.00±2.00	00.00

The highest inhibition and killing potentiality was observed when the extraction method was done by using the combination of the two solvents; methanol and chloroform in ratio 1:1(v: v), where the results recorded in table (3) showed the increase in inhibition zones with low concentrations when compared with the positive control and with the two previously mentioned data of the present study against the same studied yeast species. The combined extract exhibited the fungicidal effect against all the tested pathogens at different concentrations; 15% against *Candida glabrata* (the most sensitive isolate in the present study), 20% against *Candida tropicalis*, and 25% against *Candida kefyr* and *Candida albicans* (the most resistant one)

Table 3: Anti-*Candida* activity of *Ricinus communism* mixture (combination of methanol and chloroform 1:1v/v) extract in concentrations (5, 10, 15, 20 and 25%) against *Candida* species (*tropicalis*, *kefyr*, *glabrata*, and *albicans*) respectively depending on inhibition zone diameters (mm)

Candida species	combined extract concentrations					Clotrimazole	DMSO 20%
	5%	10%	15%	20%	25%		
<i>C. tropicalis</i>	38.33±5.69	46.67±7.09	64.67±1.53	80.00±0.00	80.00±0.00	51.67±0.58	00.00
<i>C. kefyr</i>	22.33±3.51	36.67±1.15	47.33±2.89	62.33±5.03	80.00±0.00	46.33±1.53	00.00
<i>C. glabrata</i>	49.33±0.58	60.33±3.79	80.00±0.00	80.00±0.00	80.00±0.00	53.67±3.79	00.00
<i>C. albicans</i>	17.67±0.58	27.67±4.62	38.67±3.06	54.00±1.73	80.00±0.00	33.00±2.00	00.00

The minimum inhibitory concentrations were determined by depending on the studied *Candida* isolates growth in different concentrations from combined extracts, the data in table (4) showed the MIC values which were (13, 18, 22, and 24)% for *Candida glabrata*, *tropicalis*, *kefyr*, and *albicans* respectively.

Table 4: Minimum inhibitory concentration (MIC) values of combined plant extract against *Candida* species (*tropicalis*, *kefyr*, *glabrata*, and *albicans*) in 24h incubation period.

Candida species	Combined extract Concentrations				
	(10-14)%	(15-19)%	20-24	Clotrimazole	DMSO 20%
<i>C. tropicalis</i>		18		+	+
<i>C. kefyr</i>			22	+	+

<i>C. glabrata</i>	13			+	+
<i>C. albicans</i>			24	+	+

(+)growth

(-)no growth

Discussion

All the data mentioned in the present study refer to three results; firstly; the differences in *Candida* isolates susceptibility to all plant extracts which can be attributed to genetic variations among them which may be expressed as virulence factors [28], that especially noted with *C. albicans* which was the most resistant isolate in the present study, secondly, the differences in extraction process efficiency depending on the organic solvent that used in extraction method; the high extraction activity of the third extract can be as a result of the polarity index which plays an active role in the extraction process. Many studies revealed that the extraction of different organic solvents has significantly different antimicrobial activity depending on their polarity which has effects on the quantity and quality of phytochemical compounds in crude extracts [29-32]., and thirdly the gradual improvement in extract potency in conjunction with the increase in extract concentration that may be a consequence due to the increase in phytochemical content in every sequential treatment as proved in MIC values of the combined extract which were 13, 18, 22, and 24 for *Candida species (glabrata, tropicalis, kefyi, and albicans)* respectively. All mentioned results of present study can be consoled to the presence of phytochemical compounds in *Ricinus communism* leaves which responsible about its antimicrobial activity [29], these results agreed with previous studies that confirmed *Ricinus communism* extracts have different bioactivity [29, 33].

Conclusion

The extract showed anti-fungal activity that can be used as a safe alternative of antibiotics especially for resistant strains.

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