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EFFECTIVE ANTIFUNGAL COMPOUNDS OF *LACTOBACILLUS CASEI* AGAINST VULVOVAGINAL CANDIDIASIS FOR WOMEN IN THI-QAR GOVERNORATE

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Abstract

One hundred fifty four samples were collected from Bint Al-Huda Teaching Hospital in Thi- Qar divided to 104 women infected urinary tract infection (UTI) and 50 healthy women as control group, the result revealed that 48 patient infected with vulvovaginal candidiasis**,** *L.casei* (ATCC39392) extracted and chemical compounds identified by Gas Chromatography, the extract of *L. casei* inhibited *C. albicans* in well diffusion assay and the inhibition zone, its was 15 mm, while minimum inhibiter concentration was 32 µg/ml , examine effected of extract as biofilm showed that 20.8% of isolates formed strong biofilm while moderate and weak 70.8% and 8.3% respectively, the extracted have significant effect as antibiofilm, it was able to reduce biofilm strongly 60% , moderate 20% while there was 20% not effected, examination cytotoxicity of this extract showed that there is low toxicity on normal cells of dermal cell line and hepatic cell line, Ic50 was and 192.7µs/ml for both WRL-68 and HdFn cell lines, which inhibition rate 14.2 and 16.1 for HdFn and WRL-68 respectively at concentration 400 µg /ml.

Key words: Vulvovaginal candidiasis, *Lactobacillus casei* , Probiotics**,** Antifungal

Introduction

The most frequent opportunistic yeast infection caused by *Candida* species is called vulvovaginal candidiasis, and Candida albicans primarily cause it. It is a component of the vaginal tract's normal microbiota. Vaginal infections are prevalent in women who can bear children. (Senthilganesh *et al*., 2022)(Al Fayyadh, & Al-Yasiri, 2021) that are brought on by a sexually transmitted infection or a condition that causes the natural microflora to proliferate, including infections brought on by *Candida albicans* (Waheeb, 2023). Approximately 75% of fertile women experience this at some point in their lives. (Sun *et al*., 2023) and 20% of women suffer an illness each year. (Hellier& Wrynn, 2023). It is important to note that a secondary episode of Candida infection may occur among 40-50% of individuals, while a significant proportion of 20-50% may remain asymptomatic. Common symptoms of candidiasis include malodorous vaginal discharge with a characteristic texture resembling cottage cheese, accompanied by itching, burning, and vulval erythema. The duration of candidiasis untreated is typically associated with the severity of symptoms. It is therefore imperative to seek timely intervention to minimize the impact of candidiasis on the affected individual's health and quality of life 2021)
(Waheeb et al., 2022)(Venugopal *et*

Lactobacillus casei is a probiotic bacteria with several health. It is a nonpathogenic, Gram-

positive lactic acid bacteria that is present in the human microbiome. The antitumoral action of *L. casei* has been investigated, and it has been connected to processes such as immune response modulation, activated macrophages, and control of cell death. (Fichera et al., 2016). This work, identified the types of antifungal substances found in culture supernatants that previously demonstrated effective in vitro. *Candida albicans* are commensal colonizers of the human body such as the vagina. On the other hand, *Lactobacillus casei* is a probiotic bacteria that can interact with *Candida albicans* and other microorganisms in the host microbiota. *Lactobacillus casei* has been shown to have inhibitory effects on *candida albicans*. This inhibition is possibly related to the production of lactic acid (Paniágua et al., 2021). Antifungal drugs are certainly required to treat. This condition and the infected devices generally need to be removed(Saadaldin, 2011). This study was conducted to explain the evaluation inhibition of the potential of secondary metabolites of *L.casei* on *candida albicans*.

Materials and Methods

The samples were collected from a group of patients at Bint Al-Huda Teaching Hospital in Thi-Qar, and the control groups were collected from healthy and & nonpregnant women during the period between August to November 2023. It included 154 subjects (50) control range of age from 19- 52 years 63 patients with pregnant women (17-46 years) and 41 patients with non-pregnant women (22- 48 years).

All collected samples were identified by culturing on the Sabouraud Dextrose Agar. Positive samples were examined with germ tube test and performed according to (Arafa et al., 2023), using human serum and incubation at 37 C for 3 h. It was confirmed on chromogenic agar candida, the colors of the developing colonies on the medium were monitored, and the colors of each colony were used to distinguish yeast species compared to the standard (Hajjeh et al., 2004). The isolates are then examined with a Vitek2 compact system. *L. casei* (ATCC 39392) obtained from the Center of Biotechnology Research/Al-Nahrain University as stander ATCC culture. The active compound was extracted from the culture for 72h and then the growth filter was centrifuged at 1000rpm/15min to separate the supernatant. The active constituted of this extract was identified by GC-mass. An inhibition assay was conducted to study the effect of ingredients produced by *L.casei* on *C. albicans* according to (Abbas et al.,2018). The minimum inhibitory concentration was performed to identify the concentrations of secondary products of *L. casei* on *C. albicans*. Biofilm examination was performed using the microtiter method of OD 630nm for the 48 *C. albicans* isolates (Wu et al., 2020. Anti-biofilm examination of *Lactobacillus casei* extract was performed for all strong Candida albicans (Aydin et al., 2019). Toxicity assay was conducted to examine the effectiveness of a product derived from *L.casei* by MTT assay MTT assay (in vitro cytotoxicity) The cytotoxic activity of AKBA was assessed using an MTT assay against HdFn and WRL-68 cells. Cells were seeded into a flat 96-well plate (100 µL per well at a concentration of 1 × 106 cells per well) and allowed to proliferate for 24 h at 37 °C in a CO2 (5%) incubator. Following incubation, the medium was replaced by a fresh medium containing increasing treatments of AKBA (12.5, 25, 50, 100, 200, and 400 µg mL−1) and was

added for each well. The plate was further incubated for another 24 hours. Following incubation, 10 µL of MTT solution was added to each well. The plate was incubated for another 4 h. The medium and MTT were discarded, and 100 µL of solubilization solution (DMSO) was added to solubilize the formazan crystals. When the solubilization process of purple formazan was complete, optical density at 570 nm was measured using a plate reader (Bio-Rad, USA), and inhibition percentage was calculated concerning vehicle control (untreated cells). Treatments were done in triplicate, and a half-maximal inhibitory concentration (IC50) value was calculated for each cell line (Ahmed et al., 2023).

Results

Forty-eight isolates of *C. albicans* were identified by Sabouraud Dextrose Agar, chromogenic agar *candida* germ tube method, and Vitek 2 system. The findings suggest that the active ingredient extracted from *L.casei* may offer a natural and potentially effective alternative for combating *C. albicans* infections. The search results did provide specific details about the active ingredient extracted from *L.casei* as in Figure (2).

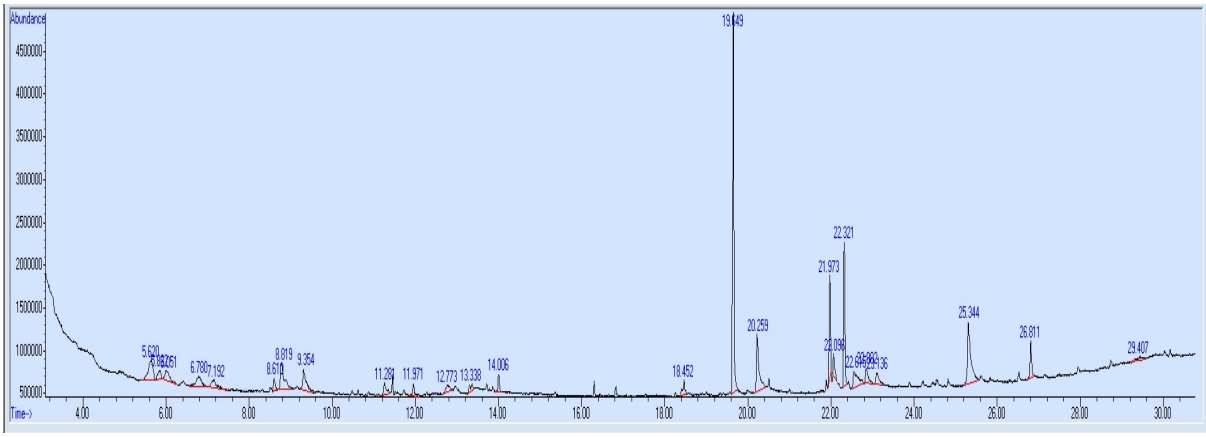

Figure (2): GC-mass diagram of *L.casei* extract

The search results provide information on the antifungal activity of various compounds against *C. albicans*, including the active compounds) that were identified by GC-mass, as shown the Table (1).

Table (1): Active Compounds of *L.casei* identified by GC-mass

NO.	Compound	Gas number
1	Benzoic acid, methyl ester	16307 000093-58-3 42
2	1,2-Hydrazinedicarboxaldehyde	1947 000628-36-4 42
3	2-Imidazolidinone, 1,3-dimethyl-	7114 000080-73-9 46
4	l-Alanine, N-iso butoxy carbonyl-, isohexyl ester	121748 1000313-44-3 50

5	Sydnone, 3,4-dimethyl-	7080 004007-18-5 53
6	1,2,4-Triazino[5,6-E]	39928 1000267-85-6 43
7	Hexadecanoic acid, methyl ester	119400 000112-39-0 99
8	n-Hexadecanoic acid	107549 000057-10-3 50
9	7-Hexadecyn-1-ol	92513 000822-21-9 64
10	9-Octadecenoic acid (Z)-, methyl ester	141302 000112-62-9 99
11	Methyl stearate	143126 000112-61-8 99
12	Oleic Acid	129337 000112-80-1 52
13	9-Octadecenamide, (Z)-	128446 000301-02-0 89

The antifungal results revealed that the extracted *L.casei* have antifungal properties and inhibit the growth of *C. albicans* and show an inhibition zone ranging about (15mm).

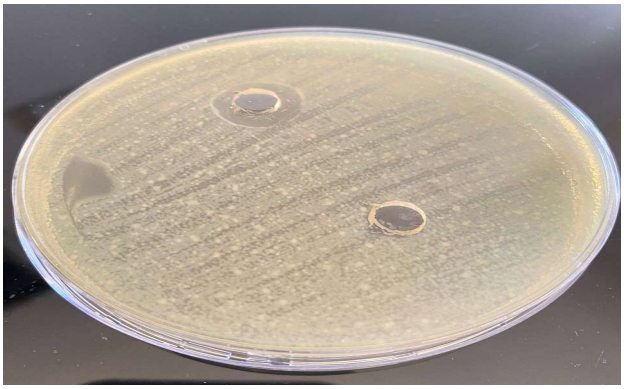

Figure (1): inhibition zone of *L.casei* on *C.albicans*

Minimum inhibitory concentration of the effective products of *L.casei* against *Candida albicans* was conducted and it was found that the concentration of 32 µg/ml is the lowest concentration that inhibits the growth of *C. albicans*.

A biofilm test was conducted for the 48 isolates of *C. albicans*, the present study declared that out of 48 *Candida albicans*, four isolates formed a weak biofilm, thirty-four isolates formed a moderate biofilm and ten isolates formed a strong biofilm shown in table (2)

Table (2): Biofilm intensity based on estimated cutoff value*of *C. albicans* isolates

ID Biofilm	Intensity	Number of isolates	percentage) %
1	Non-biofilm producer	0	0%
2	Weak	4	8.33%
3	Moderate	34	70.83%
4	Strong	10	20.84%

cutoff value = 0.0629 (defined as the Mean of control OD630 plus 3 Standard deviation).

The inhibitory effect of the active products of *L.casei* was studied in Sub MICs concentration on *C. albicans* biofilm formation. Antibiofilm test was conducted for the ten isolates of *C. albicans* strong biofilm isolates, the present study declared that the extract of *L.casei* reduced significantly by 60%, while affected moderately on 20% of isolates of biofilm of isolates also 20% isolates didn't effected by this treatment. sensitivity to the active products of *L.casei* and the biofilm formation was reduced as shown in Figure (3).

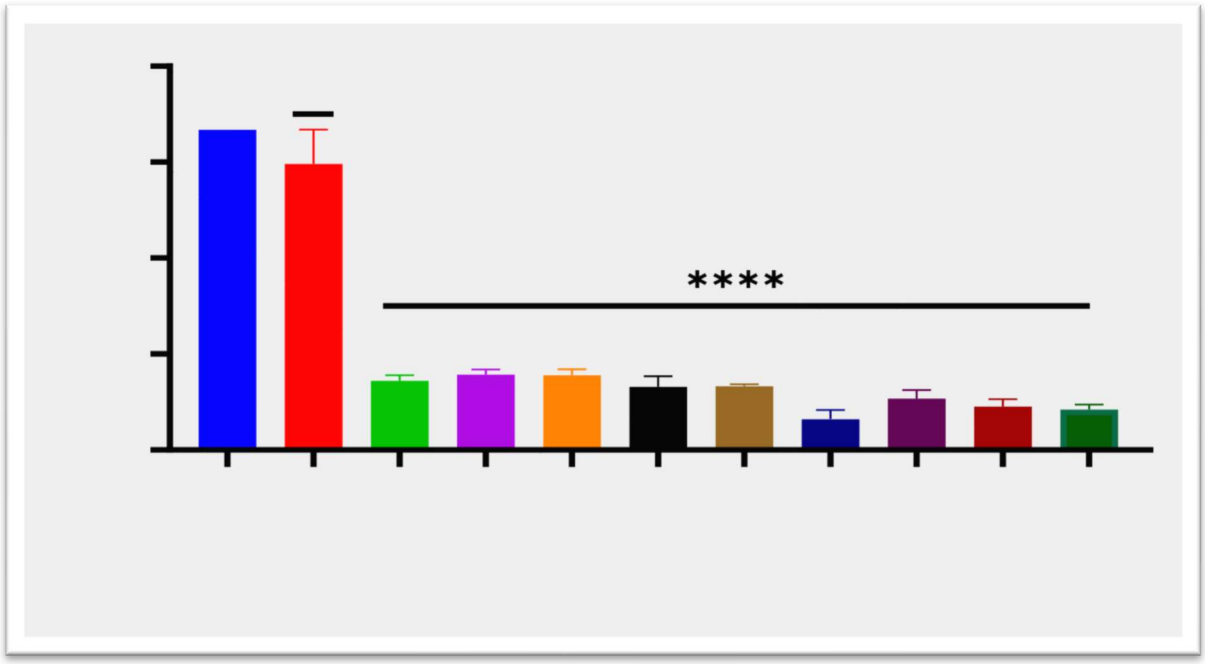

Figure (3): Antibiofilm activity of *L.casei* sub-MIC

The extract of *L.casei* was examined for its toxicity in the human normal dermal fibroblast cell line (HdFn) and on the hepatic cell line (WRL-68). The results revealed that the extract has very low toxicity on both cell lines and the Ic50 was 122.5 and 192.7 µg/ml on WRL-68 and HdFn

respectively. As shown in Figure (4)

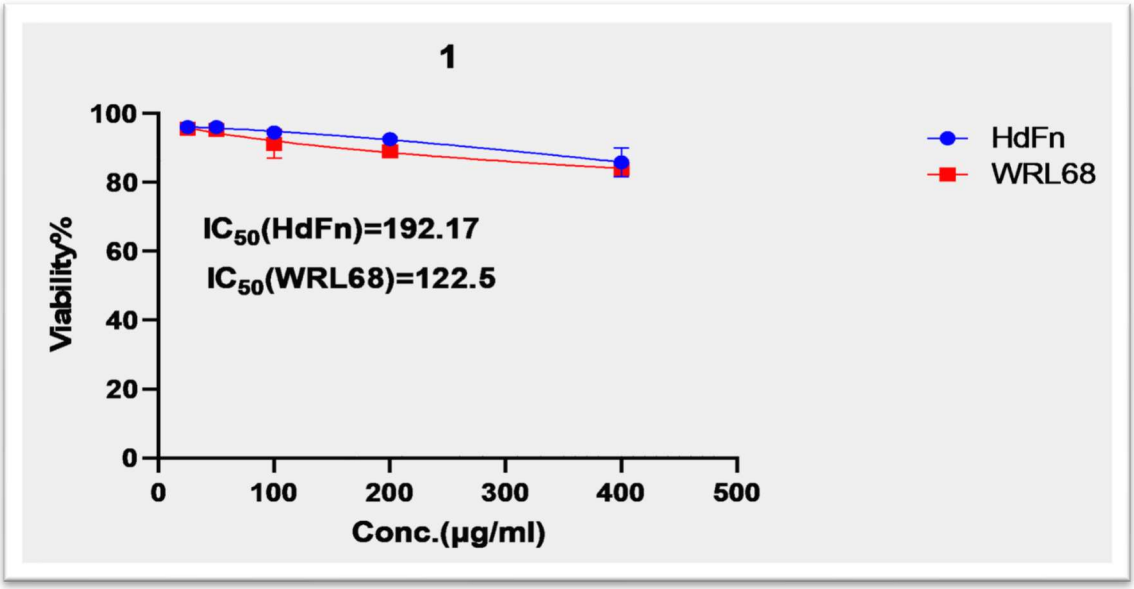

Figure (4): Cytotoxicity of *l.casei* extract on HdFn and WRL-68 cell line.

While the inhibition rate ranged from 14.2-4% and 16.1- 4.5% for HdFn and WRL-68 respectively in concentration between 400 – 25 µg /ml. as shown in the table. (3).

Table (3): The cytotoxic effect of extract of *L.casei* on HdFn and WRL68 cell line

Concentration µg mL^{-1}	Mean viability (%) ± SD	
	HdFn	WRL68
400	85.8±4.1	83.9±1.7
200	92.5±1.5	89.0±1.2
100	94.4±0.5	91.1±4.2
50	96.0±0.9	95.1±0.5
25	96.0±0.6	95.5±0.8

Discussion

L. casei extract has potential antifungal properties against *Candida albicans*. That fits with (Paniágua et al., 2021) who found *L. casei* has antifungal activity against the *Candida* species. In all isolates, there's a significant effect on Candida inhibition and that may be results of Benzoic

acid which is identified by GC-mass that have an antifungal mechanism involving the Benzoate buildup, which lowers intracellular pH into the region where phosphofructokinase is sensitive, inhibits glycolysis and causes a decrease in ATP. (krebs et al., 1983). Hexadecanoic acid, methyl ester that has antifungal properties according to certain research, fatty acids and their derivatives, such as hydroxy fatty acids, have antifungal properties that cause the uncontrollable release of intracellular electrolytes and damage the fungal cell membrane. (Omoruyi et al., 2014)

Also, many researches indicate that methyl which is a fatty acid derivative can be destroyed cell membranes and it was found that methyl linoleate, a fatty acid methyl ester (FAME), showed antifungal activity (pinto et al., 2017).
Variations in the ability of isolates to form biofilms, variations in the number of primary cells that succeeded in adhering, and variations in the quantity and quality of quorum sensing signaling molecules (autoinducer) produced from each isolate are all potential causes of the variations in biofilm thickness (Di Domenico et al.,2018). This outcome is consistent with prior research that looked into the biofilm formation of *Candida albicans*. *L.casei* in this study produced different compounds that neutralize biofilm such as vitamin E that can significantly reduce in vitro biofilm formation of many results (Soltani et al., 2021). In addition, n-hexadecanoic acid is attributed to antifungal, known for its ability to disrupt the fungal cell membrane. Also, the presence of 9-Octadecenamide is not well understood, but it is believed to interact with the cell membranes of fungi, similar to natamycin, that binds to ergosterol in the cell membranes of fungi (Kim et al., 2019).
Its antimicrobial and antifungal activities suggest that it may have potential implications in the context of biofilm formation and control.

World Health Organization (WHO) and Food and Drug Administration (FDA) rules state that probiotics are generally considered safe (GRAS) and belong mostly to the genera Lactococcus and Lactobacillus. It is still necessary to verify their safety (Salminen et al., 1998) (Marteau et al., 2002). It is possible to construct the following theoretical ideas about the safety of probiotic therapy: (1) local or systemic infections, such as fungemia, bacteremia, meningitis, endocarditis, etc.; (2) metabolic and toxic disorders, such as invasion of epithelial cells, mucus layer degradation in the gut, toxin production, etc.; (3) plasmid transfer containing virulence factors and antibiotic resistance genes into the gut microbiota, which results in the formation of new clones of bacterial strains; (4) excessive immune system stimulation in susceptible individuals (Marteau et al., 2002) which threatens human health.

Conclusion

Based on the provided information, we can draw some conclusions from the study:
1. The study indicates a higher prevalence of urinary tract infection (UTI) among women in the sample compared to healthy women in the control group. This suggests that UTI is common among the women in the study population.

2. Chemical compounds were extracted from L. casei (ATCC39392) using gas chromatography. The study showed that this extraction has an inhibitory effect on the fungal organism C. albicans in the well diffusion assay, with an inhibition zone diameter of 15 mm. The minimum inhibitory concentration (MIC) of the extract was determined to be 32 µg/ml. This indicates that the extract has effective inhibitory activity against C. albicans.

3. The study also showed that the extract has a moderate effect on biofilm formation by C. albicans. The results found that 20.8% of the isolates formed strong biofilms, while 70.8% and 8.3% formed moderate and weak biofilms, respectively. The extract exhibited a moderate antibiofilm effect, reducing biofilm formation by 60% for strong biofilms and 20% for moderate biofilms, while it did not affect 20% of the isolates.

4. Regarding the extract's cytotoxicity, the study demonstrated low toxicity on normal dermal and hepatic cell lines. The IC50 value was calculated as 192.7 µg/ml for both the WRL-68 and HdFn cell lines. The inhibition rates were 14.2% and 16.1% for HdFn and WRL-68, respectively, at a concentration of 400 µg/ml. This suggests that the extract has low toxicity on normal cells.
In summary, the study indicates that the extracted compounds from L. casei have inhibitory effects against C. albicans, moderate antibiofilm activity, and low cytotoxicity on normal cells.

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