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Antifungal effect of intimate gel based on hydro-ethanolic extract of *Cyperus esculentus* L. and probiotic bacteria in Wistar rat.

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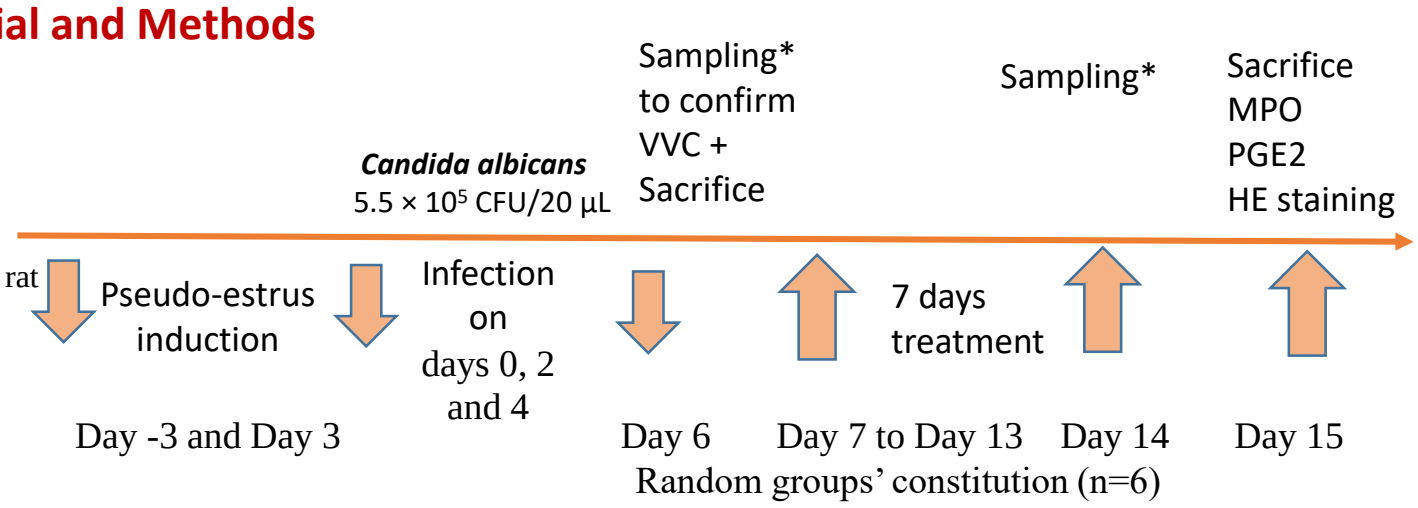
Introduction

Currently, scientists are increasingly interested in women's health, particularly focusing on the vaginal microbiome.Vulvovaginal candidiasis (VVC) is an infection caused by fungi of the genus *Candida* on the vaginal mucosa. In some cases, it can become pathogenic. It manifests itself as leucorrhoea, vulvar hyperaemia, intense pruritus, dysuria and dyspareunia, and affects around 75% of women at least once in their lives. The conventional treatment recommended for vaginal infections caused by *C. albicans* is antifungal therapy include azoles. However, the prolonged use of antibiotics increases relapse rates, likely due to the inability to restore the normal balance dominated by *Lactobacillus*. Our aim is to propose an effective and natural solution for combating VVC. An intimate gel based on tiger nut extract and microencapsulated strain of *Lacticaseibacillus rhamnosus* SL42, was studied as a novel treatment for candidiasis in œstrogenic-Wistar rat as an animal model of VVC. Under some issues' health consequences, the drug uses would be harmful or not preconized by doctor (i.e, pregnancy, Recurrent VVC, host immunity issues…), that why natural and effective preparation would be an alternative for minimizing discomfort adjacent to VVC or the healing process.

Material and Methods

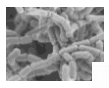
♀(150-200g, Wistar rat n=48)



The experimental scheme

*= *C. albicans* load in vaginal lavage

Treatment groups:



+SL42 : NCBI GenBank N°OQ300076 → In Xanthan beads, 10^8 CFU/mL applied intravaginal once a day/7 days.

+Lcr35 → 10^8 CFU/mL applied intravaginal once a day/7 days.

+Econazole nitrate emulsion, 1% → 0,5 mL applied once a day/7 days.

+Intimate gel → Intimate gel ϵ 2.2 g SL42 in xanthan beads + hydro-ethanolic tiger nut extract (30/70; v/v) at 125µg/mL: 0,5 mL applied once a day/7 days.

+Tiger nut extract → Hydro-ethanolic tiger nut extract (30/70; v/v) at 125µg/mL in DMSO: 0,5 mL applied once a day/7 days.

Control groups:

VVC model → Untreated : Three separate vulvovaginal infections of *C. albicans* (5.5 × 10^5 CFU/20 µL).

Control → Uninfected : Three separate vaginal inoculation of 0,9% Saline solution

Results

Antifungal MIC and MFC measurement results.

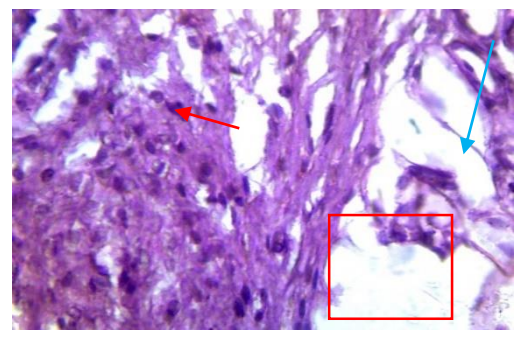
	1 mg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL	31.25 µg/mL	15.625 µg/mL	7.812 µg/mL	3.906 µg/mL	1.953 µg/mL	DMSO (-)	Econazol (+)
F	-	-	-	-	-	-	+	+	+	+	-	+
G	-	-	-	-	-	-	-	-	+	+	-	+
H	-	-	-	-	-	+	+	+	+	+	-	+

Rat groups	MPO activity (%)
Control	99 ± 2.4
Infected untreated (VVC model)	570 ± 9.2***
Infected + treated with 1% econazole nitrate	95 ± 3.2
Infected + treated with SL42	90 ± 1.44
Infected + treated with Tiger nut extract	108 ± 0.24*
Infected + treated with Lcr35	101 ± 0.70
Infected+ treated with the intimate gel	89 ± 1.88

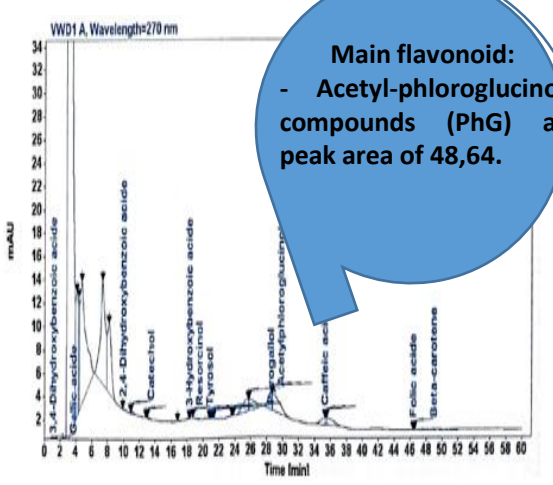
Rat groups	PGE_2 activity (pg/mL)
Control	54 ± 5.07
Infected non treated (VVC model)	806 ± 4.2 ***
Infected + treated with 1% econazole nitrate	89 ± 2.30*
Infected + treated with SL42	89 ± 1.40*
Infected + treated with Tiger nut extract	95 ± 2.66*
Infected + treated with Lcr35	82 ± 0.33*
Infected+ treated with the intimate gel	74 ± 1.09

** P≤0.05 compared to control group; *** P≤0.001 compared to control group*

Anticandidiasis effect of the intimate gel compared to the tiger nut hydro-ethanolic extract on PDA agar.

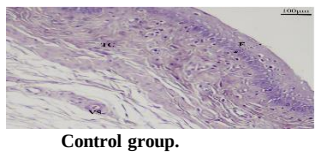


***Candida albicans* invasion in our VVC model as demonstrated by HE staining (day 5). Damaged vaginal epithelium (red box), neutrophils infiltration (red arrows) and hyphae (blue arrows). Scale bar =50µm.**

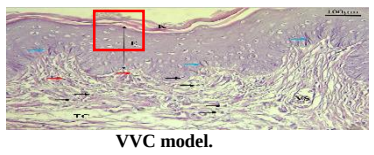


Main flavonoid:
- **Acetyl-phloroglucinol compounds (PhG) at peak area of 48,64.**

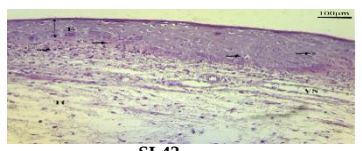
HPLC DAD/UV chromatogram of yellow nutsedge extract (*Cyperus esculentus* L.).



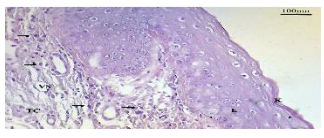
Control group.



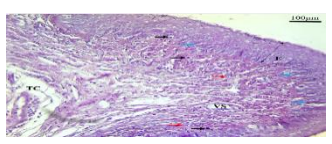
VVC model.



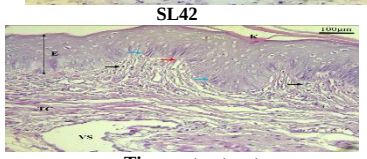
SL42



Econazole nitrate, EG 1%

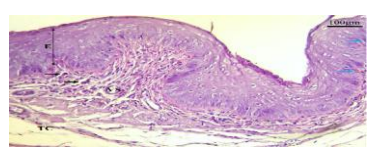


Gynophilus ® Lcr35



Tiger nut extract

Histological section (HE staining) of the vaginal tissues. VS: blood vessels, TC: connective tissue, E: epithelium, K: keratin, neutrophil infiltration of (black arrows), exfoliated vaginal epithelial cells (red box), *Candida* yeasts (red arrows) and hyphae (blue arrows).



Intimate gel

The fungal burden and number of the uninfected animals in all rat groups.

	Control	VVC model	Econazole nitrate 1%	Lcr35	SL42	Tiger nut extract	Intimate gel
One day after 7 days' treatment (day 15)	0.0±0.0 CFU/mL (10^3); 6/6 Uninfected rats	50.00±1.17 CFU/mL (10^3); 0/6 Uninfected rats	2.00±2.87 CFU/mL (10^3); 5/6 Uninfected rats	7.00±4.22 CFU/mL (10^3); 5/6 Uninfected rats	00.22±3.11 CFU/mL (10^3); 5/6 Uninfected rats	11.10± 0.33 CFU/mL (10^3); 3/6 Uninfected rats	00.77±4.22 CFU/mL (10^3); 5/6 Uninfected rats

Conclusion

The conventional treatment recommended for vaginal infections caused by *C. albicans* is antifungal therapy that includes azoles. However, the prolonged use of antibiotics increases relapse rates, likely due to the inability to restore the normal balance dominated by *Lactobacillus* in the vagina.

The data obtained highlighted that the intimate gel based on tiger nut extract and containing *L. rhamnosus* SL42 beads significantly preserved the vaginal tissue architecture and prevented vaginal inflammation. Its efficacy for the management of RVVC and reducing the adhesion of *C. albicans* was equivalent to that of the probiotic bacteria Lcr35 or Econazole nitrate. This positive action might be due to the anti-inflammatory potential of tiger nut extract's flavonoids combined with that of strain SL42 . Therefore, *L. rhamnosus SL42* has shown to be a promising probiotic for treating and preventing VVC, and it could be useful in products designed to prevent RVVC.