ORIGINAL ARTICLE

sciendo

10.2478/AMB-2023-0039

IN VITRO EVALUATION OF CORN SILK EXTRACT AS A POTENTIAL ALTERNATIVE THERAPY FOR VULVOVAGINAL CANDIDIASIS

P. Liberty[1], I. B. Putra[2], N. K. Jusuf[2], M. I. Sari[3]

[1]Medical Study Program, Faculty of Medicine, Universitas Sumatera Utara, Indonesia
[2]Department of Dermatology and Venereology, Faculty of Medicine, Universitas Sumatera Utara, Indonesia
[3]Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara, Indonesia

Abstract. Aim: *To determine the effectivity of corn silk extract in inhibiting the growth of C. albicans.* ***Materials and Methods:*** *Corn silk used in this study was Zea mays L. var. indurata and was collected from Desa Suka Makmur, Deli Serdang, Sumatera Utara Province, Indonesia. Tested C. albicans is C. albicans from VVC patients, obtained from the Microbiology Laboratory of Faculty of Medicine, Universitas Sumatera Utara. Preparation of this study included corn silk extraction by maceration method with 96% ethanol, followed by dilution with 10% DMSO (Dimethyl Sulfoxide) into concentrations of 75%, 50%, 25%, and 12.5%. Well diffusion method was used to detect growth inhibition of C. albicans and agar dilution was used to determine MIC (Minimum Inhibitory Concentration) and MFC (Minimum Fungicidal Concentration) of corn silk extract. Data were statistically analyzed with Kruskal-Wallis analysis.* ***Results:*** *From this study, corn silk extract of all concentrations showed an inhibitory effect against C. albicans. The mean diameter of inhibition zones for each concentration respectively from highest to lowest concentration were 27.4875 ± 0.3838 mm, 26.7250 ± 0.2533 mm, 25.7250 ± 0.2598 mm, and 24.9375 ± 0.2462 mm. Kruskal-Wallis analysis showed significant results (p-value 0.001). MIC and MFC were 1.5625% and 6.25%, respectively.* ***Conclusions:*** *Corn silk extract of concentrations 75%, 50%, 25%, and 12,5% have antifungal activity against C. albicans. The higher the concentration of corn silk extract, the higher the efficacy in inhibiting the growth of C. albicans.*

Key words: *Candida albicans; corn silk extract; vulvovaginal candidiasis*

Corresponding author: *Imam Budi Putra, email: imam_65@yahoo.com*

Received: *03 April 2023;* ***Revised/Accepted:*** *20 July 2023*

INTRODUCTION

Vulvovaginal candidiasis (VVC) is an infection caused by Candida species, especially *C. albicans* [1]. The incidence of VVC is high, approximately 78% of women have one episode of VVC and 34% have two or more episodes in a lifetime [2].

Over the past few years, treatment of VVC is challenging due to the increasing resistance of *C. albicans* to azoles [3]. According to previous study, 97.4% *C. albicans* was resistant to fluconazole and 94.9% was resistant to ketoconazole [4]. A study in Bulgaria showed *C. albicans* isolates were resistant to all azoles, even to echinocandins [5]. For this reason, proven alternative therapy for VVC is needed to treat azole-resistant VVC. Natural products especially plants are believed to be potential alternative therapies as they contain biologically active molecules,

one of those plants is corn [6]. Corn has silk which is widely believed to be inutile, but unexpectedly has been proven to be useful as sunblock [7]. Corn silk also has antidiabetic activity [8]. Corn silk contains alkaloids, flavonoids, terpenoids, and tannins [9]. Those phytochemical compounds are known for their antifungal effects [10, 11, 12, 13]. Thus, corn silk may be a potential alternative therapy for VVC.

This study aims to determine the effectivity of corn silk extract in inhibiting the growth of *C. albicans* with the expectation that corn silk may be an alternative therapy for VVC.

MATERIALS AND METHOD

This study was conducted at the Microbiology Laboratory of Faculty of Medicine at Universitas Sumatera Utara. The protocol of this study has obtained approval from the Research Ethics Commission of Universitas Sumatera Utara /H. Adam Malik General Hospital, Medan, Indonesia with ethical clearance number 98/KEP/USU/2021.

Materials

Corn silk used in this study was Zea mays L. var. indurata and was collected from Desa Suka Makmur, Deli Serdang, Sumatera Utara Province, Indonesia. The corn silk used in this study had previously been identified by Herbarium Medanese with identification result number 6539/MEDA/2021. Moldy and dried-out corn were excluded from this study. Tested *C. albicans* is *C. albicans* from VVC patients, obtained from the Microbiology Laboratory of Faculty of Medicine Universitas Sumatera Utara. Contaminated culture was excluded from this study.

Preparation of Corn Silk Ethanol Extract

Corn silk collected was air-dried and ground into 400 g powder, then extracted by maceration method with 96% ethanol. Extraction was more effective if the ground particle was finer [14]. After 24 hours of maceration process, the maceration solution was filtrated and evaporated to obtain thick corn silk extract. Dilution using 10% DMSO was done to attain corn silk extract of concentrations 75%, 50%, 25%, and 12,5% [15].

Inhibitory Assay of Corn Silk Extract on Growth of C. albicans

Inhibitory assay of corn silk extract on growth of *C. albicans* was done with well diffusion method. The assay was preceded by culture identification and confirmation using conventional tests. *C. albicans* from the culture was suspended in sterile distilled water and adjusted to 1×106 colony forming units (CFU/mL) (0.5 McFarland standard) using a nephelometer, then the suspension was streaked on Sabouraud Dextrose Agar (SDA) plates using sterile swabs. The number of samples (SDA plates) was determined using Federer's formula. Federer's formula is (t-1) (n-1) ≥ 15, where the t value was the number of test groups and the n value was the number of samples required. A total of 4 samples (SDA plates) were required. On every SDA plate, 6 wells of diameter 6 mm were made. Each well was introduced to different test groups which were divided into 6 test groups as followed, group I (positive control using fluconazole), group II (negative control using DMSO solution), group III (75% corn silk extract), group IV (50% corn silk extract), group V (25% corn silk extract) and group VI (12,5% corn silk extract). The SDA plates were then incubated at 37 oC for 24 hours. Inhibition zones formed after the incubation period were measured using a Vernier caliper in millimeters [16].

MIC Determination

The MIC of corn silk extract was determined by agar dilution method using SDA. Dilution of corn silk extract was needed beforehand by using two-fold serial dilution with starting concentration determined by the lowest concentration that still showed inhibitory activity in well diffusion method. SDA plates were prepared by incorporating the diluted corn silk extract into molten test agars, with each plate containing a different concentration of corn silk extract. A total of 32 inocula were added to each plate. The inoculated plates were left to dry at room temperature for no more than 30 minutes, then incubated at 37oC for 24 hours. Growth of the inocula was observed to determine the MIC. This procedure was conducted in quadruplicate [17].

MFC Determination

The MFC of corn silk extract was determined by streak plate method. The streak plate method was conducted by transferring part of the agar which concentration showed no growth of *C. albicans* in the agar dilution method into sterile SDA. Incubation was conducted at 37ºC for 24 hours. Growth of inocula was observed to determine the MFC. This procedure was conducted in quadruplicate [18].

Methods: Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (IBM SPSS Statistics 25.0). Statistical analysis was performed by Kruskal-Wallis analysis followed by Post Hoc Mann-Whitney test. The result is considered as statistically significant if p-value < 0.05.

RESULTS

Inhibitory assay of corn silk extract on growth of C. albicans

The inhibitory effect was determined by the diameter of inhibition zones formed. The results of the diameter measurement are presented in Table 1. The diameter of the inhibition zones formed can be seen in Figure 1.

The diameters of inhibition zones formed by all concentrations of corn silk extract were larger than the ones formed by positive control (fluconazole). The diameter of inhibition zones was directly proportional to the concentration of corn silk extract. The smallest diameter of inhibition zones (24.9375 ± 0.2462 mm) was formed by the smallest concentration of corn silk extract (12.5%), followed by ascending concentration to the largest concentration of corn silk extract (75%) where the largest diameter of inhibition zones (27.4875 ± 0.3838 mm) was formed.

MIC determination

The MIC determination was performed using a two-fold serial dilution of corn silk extract to attain concentrations of 12.5%, 6.25%, 3.125%, and 1.5625%. After an incubation period, growth of inocula was observed and MIC was read as the lowest concentration of corn silk extract that showed an 80% or greater reduction in growth as compared to the control [17]. Results of growth observation are presented in Table 2 and can be seen in Figure 2. The MIC is corn silk extract of concentration 1.5625%.

Table 1. Results of inhibition zones measurement

Interventions		Diameter of Inhibition Zone (mm)				Mean (mm)	Mean ± SD
		Replication					
		I	II	III	IV		
Concentration of corn silk extract (%)	75	27.7	27.05	27.9	27.3	27.4875	27.4875 ± 0.3838
	50	26.65	26.6	27.1	26.55	26.7250	26.7250 ± 0.2533
	25	25.65	25.65	26.1	25.5	25.7250	25.7250 ± 0.2598
	12.5	24.9	25.25	24.95	24.65	24.9375	24.9375 ± 0.2462
Fluconazole (positive control)		17.5	16.7	20.55	24.4	19.7875	19.7875 ± 3.4939
DMSO (negative control)		0	0	0	0	0	0

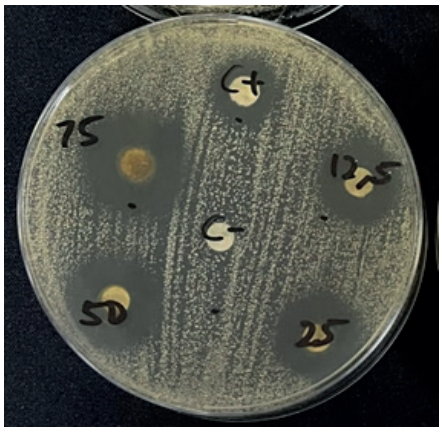


Fig. 1. Inhibition zones formed in well diffusion method: C+ refers to positive control (Fluconazole), C – refers to negative control (DMSO), 75 refers to concentration 75%, 50 refers to concentration 50%, 25 refers to concentration 25% and 12.5 refers to concentration 12.5%

Table 2. Results of growth observation in agar dilution method

Concentration of corn silk extract (%)	Growth observation			
	Replication			
	I	II	III	IV
12.5	-	-	-	-
6.25	-	-	-	-
3.125	-	-	-	-
1.5625	-	-	-	-
Positive control	+	+	+	+
Negative control	-	-	-	-

Note: (-): no growth of *C. albicans* was observed; (+): growth of *C. albicans* was observed; Positive control: plate containing SDA inoculated with *C. albicans*; Negative control: plate containing SDA and corn silk extract not inoculated with *C. albicans*

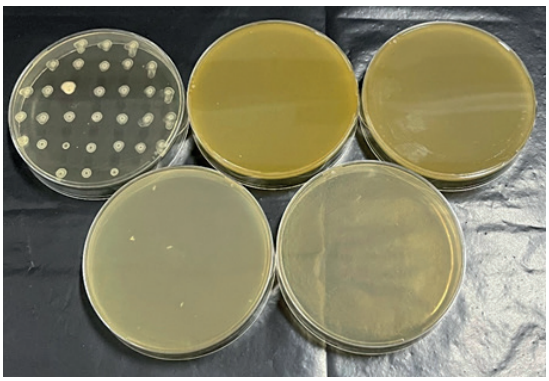


Fig. 2. Results of agar dilution method with corn silk extract concentration of 12.5% (upper middle), 6.25% (upper right), 3.125% (lower left), and 1.5625% (lower right), positive control (upper left)

MFC determination

Table 3. Results of growth observation in streak plate method

Concentration of corn silk extract (%)	Growth observation			
	Replication			
	I	II	III	IV
12.5	-	-	-	-
6.25	-	-	-	-
3.125	+	+	+	+
1.5625	+	+	+	+
Positive control	+	+	+	+
Negative control	-	-	-	-

Note: (-): no growth of *C. albicans* was observed; (+): growth of *C. albicans* was observed; Positive control: plate containing SDA inoculated with *C. albicans*; Negative control: plate containing SDA and corn silk extract not inoculated with *C. albicans*

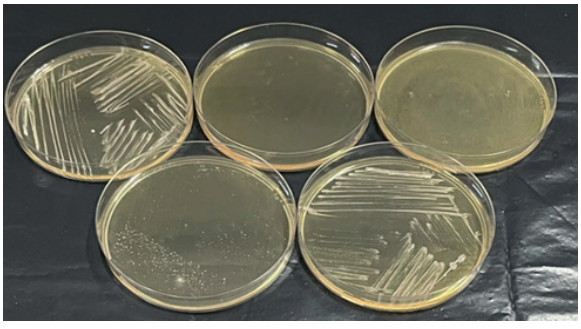


Fig. 3. Results of streak plate method with the upper ones are positive control, concentration of 12.5%, 6.25%, and the lower ones are concentration of 3.125% and 1.5625% respectively from left to right

Statistical analysis

Kruskal-Wallis analysis showed a significant result (p-value < 0.05), interpreted as a significant effect of corn silk extract in inhibiting the growth of *C. albicans* was found. Mann Whitney test showed a significant result (p-value< 0.05), which suggests that each concentration showed significant differences in inhibiting growth of *C. albicans* compared to positive control (fluconazole).

Table 4. Result of Kruskal Wallis analysis

Null Hypothesis	Test	Sig.	Decision
There is no effect of corn silk extract in inhibiting the growth of Candida albicans	Kruskal Wallis analysis	0.001	Reject the null hypothesis

Table 5. Result of Mann Whitney test

	Sig.
Comparison between corn silk extract of concentration 75% and positive control	0.021
Comparison between corn silk extract of concentration 50% and positive control	0.021
Comparison between corn silk extract of concentration 25% and positive control	0.020
Comparison between corn silk extract of concentration 12.5% and positive control	0.021

DISCUSSION

Based on the results of this study, all four concentrations of corn silk extract showed an inhibitory effect against *C. albicans*. The higher the concentration of corn silk extract used, the larger the diameter of inhibition zones formed. This result is in agreement with a previous study which showed that the higher the concentration, the higher the efficacy in the inhibition of growth [19]. The diameter of inhibition zones formed by positive control fluconazole was smaller than those formed by corn silk extract of concentration 75%, 50%, 25%, and 12.5%. This result indicates corn silk extract concentrations of 75%, 50%, 25%, and 12.5% are superior in inhibiting the growth of *C. albicans* compared to fluconazole as standard therapy for VVC [20]. This is suspected to be caused by the resistance of *C. albicans* to fluconazole [21].

The inhibitory effect of corn silk extract (Zea mays L. var. indurata) on the growth of *C. albicans* is known to originate from secondary metabolites contained in corn silk extract such as alkaloids, flavonoids, tannins, and terpenoids which existence has been confirmed in a previous report [9]. Alkaloids cause damage to cell membrane integrity and impair mitochondrial function thus inhibiting the growth of *C. albicans* [10]. Flavonoids interrupt the formation of cell wall, inhibit RNA and protein synthesis, and disrupt plasma membrane integrity in Candida species [11].

Terpenoids inhibit DNA replication in Candida species [12]. Tannins inhibit the growth of mycelium and disrupt the integrity of cell walls and membranes in Candida species [13].

CONCLUSION

In conclusion, corn silk extract of concentrations 75%, 50%, 25%, and 12.5% have an antifungal effect against *C. albicans*. The higher the concentration of corn silk extract, the higher the efficacy in inhibiting the growth of *C. albicans*. The MIC and MFC of corn silk extract obtained from this study are 1.5625% and 6.25%, respectively.

Acknowledgments: *The authors wish to thank the Head of the Department of Microbiology, Faculty of Medicine, Universitas Sumatera Utara, for assisting in the preparation of this study.*

Funding: *No specific funding has been provided for this study.*

Disclosure Summary: *The authors have nothing to disclose.*

REFERENCES

1. Nyirjesy P, Brookhart C, Lazenby G, et al. Vulvovaginal Candidiasis: A Review of the Evidence for the 2021 Centers for Disease Control and Prevention of Sexually Transmitted Infections Treatment Guidelines. Clin Infect Dis, 2022, 74(2): S162-S168. doi 10.1093/cid/ciab1057.
2. Yano J, Sobel JD, Nyirjesy P, et al. Current Patient Perspectives of Vulvovaginal Candidiasis: Incidence, Symptoms, Management and Post-treatment Outcomes. BMC Women's Health, 2019 19(48). Available at: https://doi.org/10.1186/s12905-019-0748-8
3. Arastehfar A, Kargar ML, Mohammadi SR, et al. A High Rate of Recurrent Vulvovaginal Candidiasis and Therapeutic Failure of Azole Derivatives Among Iranian Women. Front Microbiol, 2021, 12(655069). Available at: https://doi.org/10.3389/fmicb.2021.655069
4. Monroy-Pérez E, Paniagua-Contreras GL, Rodriguez-Purata P, et al. High virulence and antifungal resistance in clinical strains of Candida albicans. Can J Infect Dis Med Microbiol, 2016, 2016(5930489): 1-7. Available at: https://doi.org/10.1155/2016/5930489
5. Hitkova H Y, Georgieva D S, Hristova P M, et al. Antifungal Susceptibility of Candida albicans Isolates at a Tertiary Care Hospital in Bulgaria. Jundishapur J Microbiol, 2019, 12(7):e92079. Available at: https://doi.org/10.5812/jjm.92079.
6. Tian S, Sun Y, and Chen Z. Review Article: Extraction of Flavonoids from Corn Silk and Biological Activities In Vitro. J Food Qual, 2021, 2021(1): 1-9. Available at: https://doi.org/10.1155/2021/7390425
7. Kim Y, Cho A, Kwon S, Kim, et al. Potential Photoprotective Effect of Dietary Corn Silk Extract on Ultraviolet B-Induced Skin Damage. Molecules, 2019, 24(14): 2587. Available at: https://doi.org/10.3390/molecules24142587
8. Sheng L, Chen Q, Di L, et al. Evaluation of Anti-Diabetic Potential of Corn Silk in High-Fat Diet/ Streptozotocin-Induced Type 2 Diabetes Mice Model. Endocr Metab Immune Disord Drug Targets, 2021, 21(1): 131-138. Available at: https://pubmed.ncbi.nlm.nih.gov/32504506/
9. Rajeshwari H, Sivapriya T. Analysis of nutrients, phytochemicals, antioxidant and antimicrobial activity of corn silk extract (Zea mays L. Stigma). Int J Health Allied Sci, 2021, 10(4): 275-279. doi 10.4103/ijhas.IJHAS_148_20}
10. Dhamgaye S, Devaux F and Vandeputte P. Molecular Mechanisms of Action of Herbal Antifungal Alkaloid Berberine, in Candida albicans. PLoS One, 2014, 9(8): 104554. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4126717/
11. Aboody MSAl and Mickymaray S. Anti-fungal Efficacy and Mechanisms of Flavonoids. Antibiotics, 2020, 9(2): 45. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7168129/
12. Isah MB, Tajuddeen N, Umar MI, et al. Terpenoids as Emerging Therapeutic Agents: Cellular Targets and Mechanisms of Action Against Protozoan Parasites. Stud Nat Prod Chem, 2018, 59: 227-250. Available at: https://doi.org/10.1016/B978-0-444-64179-3.00007-4
13. Zhu C, Lei M, Andargie M, et al. Antifungal Activity and Mechanism of Action of Tannic Acid Against Penicillium digitatum. Physiol Mol Plant Pathol, 2019, 107: 46-50. Available at: https://doi.org/10.1016/j.pmpp.2019.04.009
14. Lambov N, Rachev D. Drug Technology. Phytopreparations.Sofprint Group Ltd., Sofia 2019, 523-654 cited in Tsanova-Savova S, Velikov S, Paneva S, et al. Comparative Evaluation of the Content of Antioxidant Polyphenolic Compounds in Selected Bulgarian Medicinal Plants. Acta Medica Bulgarica. 3922;49(1): 26-34. Available at: https://doi.org/10.2478/amb-2022-0005
15. Departemen Kesehatan Republik Indonesia (2017). Farmakope Herbal Indonesia edisi II. Kementrian Kesehatan Republik Indonesia. Jakarta. 615(1), pp.531.
16. Balouiri M, Sadiki M, Ibnsouda SK. Methods for In Vitro Evaluating Antimicrobial Activity: A review. J Pharm Anal, 2016, 6(2): 71-79. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5762448/
17. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically; Approved Standard – ninth edition, CLSI document M07-A9. CLSI, 2012, 32(2): 13-16.
18. Oshim IO, Desmond CO, Nwobu RAU, et al. Kinetics of Minimum Inhibitory Concentration, Minimum Bactericidal Concentration, and Minimum Fungicidal Concentration of Vernonia amygdalina (bitter leaf) on Microorganisms Isolated from Wound Infections. Int J Surg, 2016, 5(1): 8-14. Available at: doi:10.5923/j.surgery.20160501.03
19. Feng X, Wang L, Tao M, et al. Studies on Antimicrobial Activity of Ethanolic Extract of Maize Silk. Afr J Microbiol Res, 2012, 6(2): 335-338. Available at: DOI: 10.5897/AJMR11.974
20. Nabila VK, Putra IB. The Effect of Aloe Vera Ethanol Extract on the Growth Inhibition of Candida albicans. Med Glas (Zenica), 2020, 17(2): 485-489. Available at: doi: 10.17392/1098-20.
21. Sobel JD and Sobel R. Current treatment options for vulvovaginal candidiasis caused by azole-resistant Candida species, Exp Opin Pharmacother, 2018, 19(9): 971-977, doi: 10.1080/14656566.2018.147649