



The management of Vulvovaginal Candidiasis (*Kaba Yoni*) by Siddha herbo-mineral formulation *Linga Chenduram* through In-Vitro study

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ABSTRACT

Background: Candidiasis are common oral and genital infection in humans and nowadays candida species have shown resistance against various synthetic medicines. *Linga Chenduram* is mentioned in Siddha classical literature and indicated for *Mega Noi* (Sexually Transmitted Disease). *Linga Chenduram* was subjected to assess antifungal activity.

Methods: The antifungal activity of the sample was tested for *Candida albicans* ATCC 10231 to Determine the diameter of inhibition zone (DIZ) by disc diffusion and broth microdilution methods, Minimum inhibitory concentration (MIC), and Minimum fungicidal activities (MFC).

Result: The study results revealed that the DIZ for 100µg of Clotrimazole, 500µg, and 1000µg of sample drug is found to be 29mm, 14mm, and 17mm respectively. MIC 50 Value of *Linga Chenduram* is 281.086µg/ml.

Conclusions: So it can be concluded that *Linga Chenduram* possesses effective antifungal activity against *Candida albicans*.

Keywords: *Linga Chenduram*, Antifungal, *Candida albicans*, Siddha Medicine, *Lingam*

INTRODUCTION

Among various fungal infections, candidiasis is common in the oral cavity and genital areas in humans. (1,2,3,4,5) Nowadays candida species have shown resistance against various synthetic drugs. (6) Vulvovaginal candidiasis (VVC) presents mostly without symptoms. Symptoms like vulvar pruritus, burning sensation, increased vaginal discharge or malodor may present. Signs of VVC include vulvar erythema, oedema, fissures, and tenderness. A white scanty vaginal discharge may be present in the form of white thrush-like plaques or cottage cheese-like curds adhering loosely to the vaginal mucosa. (7)

The rate of incidence of VVC is limited because most of the cases are asymptomatic, and only a less women were gone to the hospital for treatment. In the obtained data about 72% of women of childbearing age were reported with VVC and nearly half of these women experienced a second episode with recurrent symptoms. (8, 9,10,11) Pregnant women, women having diabetes mellitus (DM), frequent oral sexual intercourse, and the use of tight synthetic underwear, usage of antibiotics are more prone to risk of getting VVC. (11)

According to the Siddha classical literature of *Yugi Vaithiya Chinthamani* following terminologies are mentioned under *Mega noi* such as *Neeradaippu*, *Sathaiadaippu*, *Vellai*, *Vettai*, *Kiranthi*, *Soolai*, *Araiyaappu*, *Pavuthiram*, *Neersurukku*, *Kalladaippu*, *Neerizhivu* that occurs due to sexual intercourse, alteration in food intake and *Kanmam*. (12, 13) According to *Yugi muni's* classification, in *Penkuri roga padalam*, the symptoms of *Kaba yoni* had been found similar to Vulvovaginal candidiasis. (14) In the Siddha system of medicine, mineral-based medicinal preparations are widely used for these types of fungal infections. However, there was no sufficient scientific evidence to prove the anti-microbial properties of mineral or herbo-mineral-based medicinal preparations. *Linga Chenduram* (LC) a herbo-mineral-based preparation mentioned in classical Siddha literature *Anuboga Vaithiya Navanitham*, indicated for *Mega Noi* (15) had been selected to evaluate the antifungal property.

AIM

To screen the antifungal activity of *Linga chenduram* against *Candida albicans* through the In-Vitro study

MATERIALS AND METHODS:

1. Preparation of *Linga chenduram*.

Table No 1: Ingredients of *Ling Chenduram* (15)

Name of Raw Drugs	Botanical / Chemical Name	Quantity
Purified <i>Lingam</i>	Cinnabar	17.5g (5 <i>varaganedai</i>)
Latex of <i>Thirugukalli</i>	<i>Euphorbia Tortilis</i> Rottler ex Ainslie	Sufficient
Flowers of <i>Utthamani</i>	<i>Pergularia Daemia</i> forsk	70g (2 <i>Palam</i>)
Flowers of <i>Vellaierukku</i>	<i>Calotropis Procera</i> W. T. Aiton	70g (2 <i>Palam</i>).

- **Authentication of raw drugs**

The collected raw drugs were authenticated by the Head, Department of Gunapadam, National Institute of Siddha.

- **Preparation process**

First, purified *Lingam* was measured and ground to powder form. Then it was ground for 12 hours (4 *Saamam*) with the latex of *Euphorbia tortilis* (*Thirugukalli*). After that, the ground mixture was made into a small disc (*villai*) and kept in sunlight for drying. Flowers of *Calotropis procera* (*Vellarukkam*) and flowers of *Pergularia daemia* (*Utthamani*) were ground together and made into a paste (*Karkam*). The dried *villai* was kept inside of the *Karkam*. Then it was placed into a small mud pot with a lid and sealed with clay-smear cloth (*Seelai mann*). Then the weight of the sealed mud pot with lid was measured and subjected to incineration process (*Pudam*) by cow dung cake of 4 times the weight of the measured mud pot weight. After that, it was allowed to cool itself and processed medicine was taken from the inside of the mud pot. Finally, ground into fine powder. (16, 17)

2. Screen the Anti-fungal Activity

- **Materials Required:**

Choose organism: *Candida albicans* (ATCC 10231)

- **Determination of the Zone of inhibition**

Organism placed potato dextrose agar plates were bored 10mm and different doses of LC as 250µg, 500µg and 1000µg and Clotrimazole were subjected to that and Zone of Inhibition was measured. (18,19,20,21, 22)

- **Determination of Minimal Inhibitory Concentration (MIC)**

The two-fold serial dilution method is used for MIC determination. Organisms placed in 96 well-cultured plates were compared to similar plates where different doses of LC such as 125 µg, 250 µg, 500 µg and 1000µg had been subjected and visual inspection was done by measuring the optical density (OD) at 630 nm using an ELISA plate reader. (18,19,20,21, 22) The growth inhibition for the test was determined by the formula:

$$\text{Percentage of inhibition} = (\text{OD of control} - \text{OD of test}) / (\text{OD of control}) \times 100$$

- **Determination of Minimal Fungicidal Concentration (MFC):**

Organisms placed in 96 well-cultured plates were compared to similar plates where different doses of LC had been subjected and incubated for 24 hours then swabbed onto potato dextrose agar plates; incubated at 37°c for 48 hours and observed for colony forming units (18,19,20,21, 22)

RESULT AND DISCUSSION:

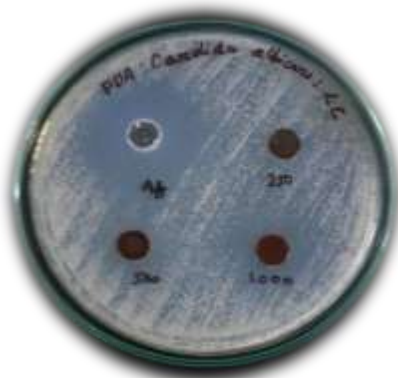
- **Determination of the Zone of inhibition**

Table No 2: Zone of inhibition measurement of sample LC and Standard

Concentration (µg/mL)	Zone of inhibition (mm)
Clotrimazole 100µg/ml	29
LC 250 µg/ml	Nil
LC 500 µg/ml	14
LC 1000 µg/ml	17

The study results revealed the zone of inhibition measured of the LC 500 µg/ml, LC 1000 µg/mL and Clotrimazole 100µg/ml such as 17mm,14mm and 29mm respectively. According to the result, the study drug LC has a low zone of inhibition measurement when compared to the standard

Figure No 1: Zone of inhibition measurement of sample LC and Standard

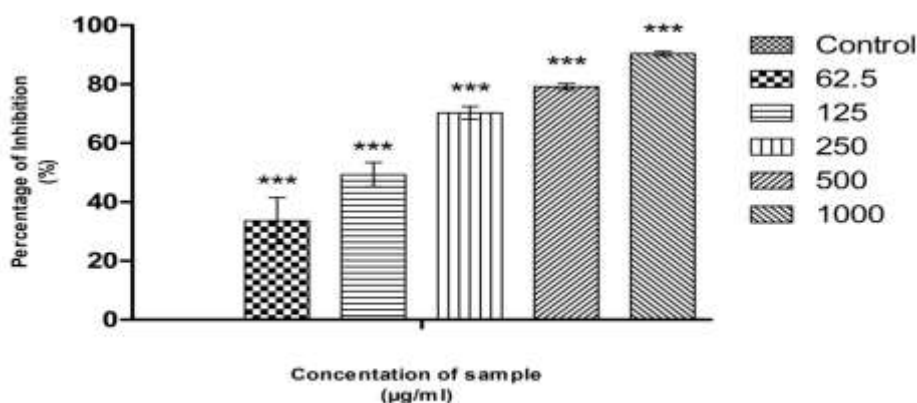


• Minimal Inhibitory Concentration (MIC) determination

Table No. 3 : The values of Minimal Inhibitory Concentration of LC and Control

Concentration	Optical Density 1	Optical density 2	Optical density 3	Average	Percentage of inhibition
Control	0.5897	0.4644	0.5301	0.5280	0
LC 62.5µg/mL	0.3167	0.3760	0.3421	0.3449	33.30
LC 125µg/mL	0.2551	0.2669	0.2721	0.2647	48.50
LC 250µg/mL	0.1508	0.1522	0.1635	0.1555	69.18
LC 500µg/mL	0.1147	0.1063	0.1066	0.1092	77.95
LC 1000µg/mL	0.0551	0.0511	0.0448	0.0503	89.10

Figure No 2 Graphical representation depicting the MIC of a sample against *Candida albicans*



The Values of the Minimal Inhibitory Concentration to the tested doses of *Linga Chenduram* and the Control group are shown in Figure No. 2 and Table No. 3. All doses of LC were more significant (***) when compared to the control group and MIC 50 Value of LC 281.086 µg/ml.

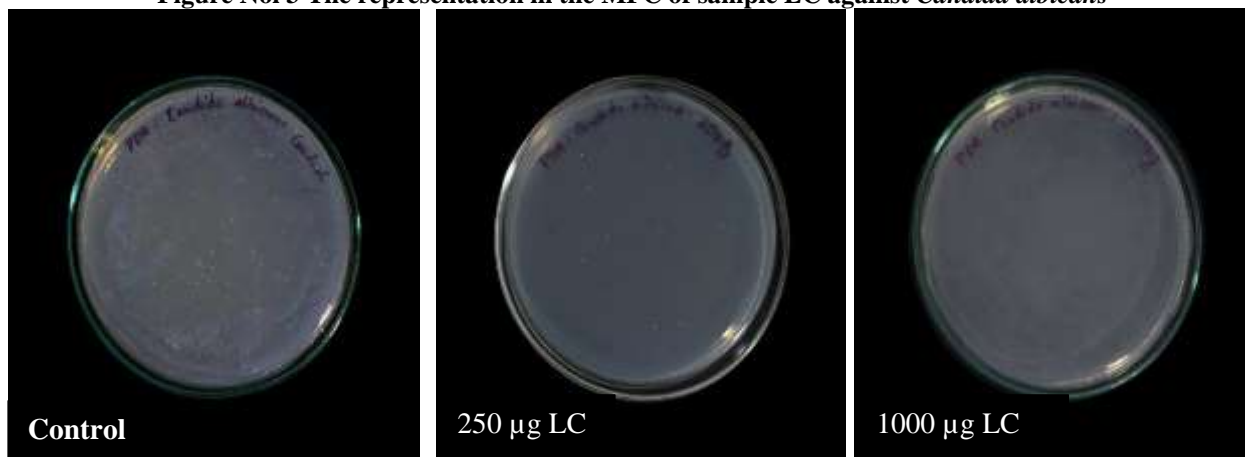
• Minimal Fungicidal Concentration (MFC) determination

According to Table No: 4, the result showed that 4.5×10^2 CFU/ml organisms were present in the 250 µg of LC and organisms weren't present in the 1000 µg of LC as shown in Table No 4 and Figure No 3. So it is concluded that *Linga chenduram* at the dose of 250 µg to 1000 µg has antifungal activity against *Candida*

Table No 4: Minimal Fungicidal Concentration of Sample LC and Control

	Number of colonies	The measure of viable clonogenic cell numbers in CFU/mL
Control (Organism alone)	32	14×10^2
LC 250 µg	9	4.5×10^2
LC 1000 µg	0	0

Figure Noi 3 The representation in the MFC of sample LC against *Candida albicans*



CONCLUSION:

According to Siddha Classical Literature and *In-Vitro* study evidence, *Linga Chenduram* possess Fungicidal activity against *Candida albicans* and a Minimal Inhibitory Concentration 50 Value of LC is found around 280µg/ml. Hence further In-vivo and clinical studies could be performed for further evaluation of the antifungal activity of *Linga Chenduram* against to *Candida albicans*.

REFERENCES:

1. Jackson BE, Wilhelmus KR, Mitchell BM. Genetically regulated filamentation contributes to *Candida albicans* virulence during corneal infection. *Microbial Pathogenesis*. 2007;42(2-3):88–93.
2. Wu TG, Mitchell BM, Carothers TS, et al. Molecular analysis of the pediatric ocular surface for fungi. *Current Eye Research*. 2003;26(1):33–36.
3. Achkar JM, Fries BC. Candida infections of the genitourinary tract. *Clinical Microbiology Reviews*. 2010;23(2):253–273.
4. Rosenbach A, Dignard D, Pierce JV, Whiteway M, Kumamoto CA. Adaptations of *Candida albicans* for growth in the mammalian intestinal tract. *Eukaryotic Cell*. 2010;9(7):1075–1086.
5. Naglik JR, Moyes DL, Wächtler B, Hube B. *Candida albicans* interactions with epithelial cells and mucosal immunity. *Microbes and Infection*. 2011;13(12-13):963–976.
6. Ksiezopolska E, Gabaldón T. Evolutionary Emergence of Drug Resistance in Candida Opportunistic Pathogens. *Genes (Basel)*. 2018 Sep 19;9(9):461. doi: 10.3390/genes9090461. PMID: 30235884; PMCID: PMC6162425.
7. Dennis Kasper, Eugene Braunwald, Stephen Hauser, Dan Longo, J. Larry Jameson, Anthony Fauci, Harrison's Principal of Internal medicine Vol 1, Mcgraw-Hill, Medical publication Division, 16 Edition, August 2004, p 768, 1185
8. Achkar J.M., Fries B.C. *Candida* infections of the genitourinary tract. *Clin. Microbiol. Rev.* 2010;23:253–273. doi: 10.1128/CMR.00076-09.
9. Spence D. Candidiasis (vulvovaginal). *BMJ Clin Evid* 2010;2010:0815.
10. Sobel JD. Genital candidiasis. *Medicine* 2014;42(7):364–368. doi: 10.1383/medc.2005.33.10.62
11. Hösükoğlu FG, Ekşi F, Erinmez M, Uğur MG. An Epidemiologic Analysis of Vulvovaginal Candidiasis and Antifungal Susceptibilities. *Infectious Microbes & Diseases*. 2022 Sep 1;4(3):131-6.
12. Anonymous, Yugimunivar Vaithya Chinthamani, Peru noi 800, Part 1, published by Committee of Siddha book Publication, 2nd Edition, 1976, P 313.
13. Sambasivam Pillai, TVS Dictionary Part I, II, III, IV, Second Edition, Published by DIM & H
14. Shanmugavelu, HPIM, Noi Naadal Noi Mudhal Naadal Thirattu, Part 1 AND 2, First Edition, Published by DIM & H.
15. Hakkim Mohammad Abdullah Sahib, Anuboga vaithiya navanitham, Part - 4, 1995 October edition, P no. 2 – 84
16. Soruban, T., S, V., & R, M. (2023)., Standardization and quality control parameters evaluation on the Siddha mineral preparation: *Linga chenduram*. *International Journal of Ayurvedic Medicine*, 13(4), 853–858. <https://doi.org/10.47552/ijam.v13i4.3138>
17. Soruban, T., S, V., & R, M. (2023)., Chemical Characterization of the *Linga Chenduram* Preparation Process as per Siddha Literature through Instrumental Analysis, *Indian Journal of Natural Sciences* 13(76), 0976 – 0997.
18. H Gopalkrishna A, M S, Muddaiah S, R S. In vitro antifungal activity of different components of *Centrathrum anthelminticum* and *Ocimum sanctum* seed oils and their synergism against oral pathogenic fungi. *J Dent Res Dent Clin Dent Prospects*. 2016 Spring;10(2):92-8. doi: 10.15171/joddd.2016.015. Epub 2016 Jun 15. PMID: 27429725; PMCID: PMC4945999.
19. B, P., K, G. S., & Kadibagil, V. R. (2022). Formulation and evaluation of antifungal activity of Kasisadi Varti against *Candida albicans*. *International Journal of Ayurvedic Medicine*, 13(2), 412–418. Retrieved from <https://ijam.co.in/index.php/ijam/article/view/2565>
20. Suresh B, Kalyanaraman VR, Dhanashekar S, Dhanraj SA, Dube R. Evaluation of certain siddha drugs in the treatment of candidiasis. *Anc Sci Life*. 1994 Jul;14(1-2):16-20. PMID: 22556670; PMCID: PMC3336504.
21. Pushkala VP, Sulekha SMP, Mathukumar S, Ragavi B, Sowmiya U. Molecular Docking Analysis of Siddha Formulation Parangipattai Chooranam Against Vaginal Candidiasis. *Appl Biochem Biotechnol*. 2022 Mar;194(3):1039-1050. doi: 10.1007/s12010-022-03813-y. Epub 2022 Jan 8. PMID: 34997904
22. National Committee for Clinical Laboratory Standards. (1993a). Performance Standards for Antimicrobial Disk Susceptibility Tests—Fifth Edition: Approved Standard M2-A5. NCCLS, Villanova, PA.