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Propulsion of Antifungal Effects of Citrullus Lanatus, Cinnamomum Verum, Murraya Koenigii on Candida Albicans- An Invitro Study

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Abstract:

Background:

The most common fungal infection is candidiasis caused by Candida albicans. It is treated either topical/systemically by a therapeutic antifungal agent, which has adverse effects when used for a long duration. Hence plant-based alternatives can be used. *Citrullus lanatus seeds, Cinnamomum verum, and Murraya koenigii* are plant-based products that can be used as an alternative to treat oral candidiasis caused by *Candida albicans*.

Aim:

The aim of the study was to evaluate the minimal inhibitory concentration of *Citrullus lanatus, Cinnamomum verum, and Murraya koenigii* against *Candida albicans*

Objectives:

The study's primary objective was to evaluate the minimal inhibitory concentration and fungicidal concentration of *Citrullus lanatus, Cinnamomum verum, and Murraya koenigii* against *Candida albicans*. The secondary objective of the study was to evaluate the biosynthesis of gold, silver, and selenium nanoparticles using *Citrullus lanatus* on *Candida albicans*

Materials And Methods:

The study was carried out in two phases. In phase one the minimal inhibitory concentration and fungicidal concentration of *Citrullus lanatus, Cinnamomum verum, and Murraya koenigii* against *Candida albicans* were evaluated. In phase two evaluate the biosynthesis of gold, silver, and selenium nanoparticles using *Citrullus lanatus* on *Candida albicans* was done.

Results:

A combination of *Citrullus lanatus, Murraya koenigii and Cinnamomum verum* had a minimal inhibitory concentration of 14 mm against fluconazole. Minimal fungicidal concentration is effective from 10^{-3} where more organisms were killed. The biosynthesis of gold nanoparticles on Citrullus lanatus greatly affects candida albicans with a minimal fungicidal concentration from $10^{-3.}$

Conclusion:

The present study shows that the combination of *Citrullus lanatus, Murraya koenigii and Cinnamomum verum* in powder form have a good effect against candida albicans. The higher the concentration greater the zone of inhibition. Biosynthesis of gold nanoparticles in *Citrullus lanatus* have a great effect against *C.albicans* with a minimal inhibitory concentration at 90%(10^{-3}).

Key Words: Candidiasis, Antifungal, candida albicans, herbal medicine, plant based products

Introduction:

Candidiasis, caused by *Candida albicans*, is the predominant opportunistic fungal infection observed in the oral cavity [1]. Usually affects immunocompromised individuals. The different types of oral candidiasis are hyperplastic or atrophic (denture) candidiasis, pseudomembranous candidiasis (thrush), linear gingival erythema, median rhomboid glossitis, and angular cheilitis. The hallmark of this condition is the manifestation of white patches on the tongue, throat, and various areas in the oral cavity [2,3]. Assessment of predisposing factors is indeed crucial in the management of candidal infections. Identifying and addressing these factors can prevent recurrent or persistent infections [4]. Usually treated by antifungals either topical/systemically by synthetic therapeutic agents [5]. Long-term use can cause side effects and interactions with a plethora of drugs taken by patients for other conditions [6]. Current research is focused on plant-based therapeutic agents with the least side effects to treat candidiasis. We need to look at plant-based products as a local drug delivery system with potential antifungal properties [7]. *Citrullus lanatus* seeds- watermelon seeds which are usually thrown away have been shown to possess antifungal, antioxidant, antimicrobial, and hepatoprotective activity [8]. M*urraya koenigii*- curry leaves used daily in cooking have the highest value in characteristic aroma and medicinal benefits [9]. The plant has been reported to have an antimicrobial, antimicrobial, and antibacterial effect. *Cinnamomum verum*, commonly known as cinnamon, serves as a spice employed in culinary practices to enhance flavor, that has antioxidant, antiviral, antifungal, anti-inflammatory, and antibacterial activity [10]. The main aim of the research was to evaluate the minimal inhibitory concentration of *Citrullus lanatus, Cinnamomum verum, and Murraya koenigii* against *Candida albicans*

Materials And Methods:

The present study is designed to evaluate the minimal inhibitory concentration of *Citrullus lanatus, Cinnamomum verum, and Murraya koenigii* against *Candida albicans*.

STUDY SETTING:

The study was carried out invitro- Laboratory setup

SRB Number- SRB/SDC/OMED-2104/21/001

SAMPLE COLLECTION:

Fresh watermelon seeds, curry leaves, and cinnamon bark sticks were collected from the local market in Chennai.

PHASE I:

PREPARATION OF THE PLANT EXTRACT:

Watermelon seeds, curry leaves, and cinnamon bark sticks were gathered, washed with distilled water, and air-dried. These samples were finely ground using a mechanical grinder. Subsequently, twenty grams of each plant powder were mixed with 100 ml of distilled water in a sterile mortar and pestle, then filtered through Whatman

NO.1 filter paper and collected in a 250 ml glass flask. The flask was sealed with cotton and stored in a refrigerator at 4˚C for 24 hours. Afterward, it was filtered again and placed in a hot oven for 5 days at 30 ˚C to allow complete solvent evaporation, resulting in a crystalline powder form. Finally, one gram of each extract was diluted with dimethyl sulfoxide (DMSO). (Fig 1).

PREPARATION OF CULTURE MEDIUM:

Freeze-dried *C. albicans* was acquired and then reconstituted by opening the ampules containing the freeze-dried microorganisms and mixing them with distilled water. This mixture was thoroughly stirred using a sterile stirrer and left to stand for thirty minutes. Each prepared mixture was aseptically applied and evenly spread on a sterile culture media plate using a swab. For *C. albicans*, Sabouraud Dextrose Agar (SDA) was autoclaved at 121°C for 15 minutes. The *C. albicans* samples were inoculated and incubated at 37°C for 2 hours. Subsequently, a cotton streak culture was performed. Four wells were created, with three for *Citrullus lanatus, Cinnamomum verum, and Murraya koenigii* extracts, and one for fluconazole (the control group). (Fig 2)

FOR COMBINATION:

SDA prepared. One well cut for a combination of *Citrullus lanatus, Cinnamomum verum, and Murraya koenigii* each 33.3%, incubated for 24 hours & checked for zone formation.

MINIMAL FUNGICIDAL ACTIVITY:

SDA prepared. Serial dilution done. 20 µl of candida incubated for 4 hours and checked for UV absorbance visually.

PHASE II:

Sabouraud Dextrose Agar prepared. 25 µg/mL Ethanolic extract *Citrullus lanatus*, synthesis of gold (Au), Sliver (Ag), and Selenium nanoparticles (SeNPs) done. 25 µg/mL Ethanolic extract *Citrullus lanatus* with 125 µM $HAuCl_4$. Control group- fluconazole. *C.albicans* inoculated and incubated at 37°C for 2 hours. Cotton swab culture streak is done. 2 wells are cut for *Citrullus lanatus* biosynthesis ($HAuCl_4$) at 25 and 100 Concentrations. 1 well cut for control group fluconazole & checked for zone formation.

MINIMAL FUNGICIDAL ACTIVITY:

SDA prepared. Serial dilution is done. 20 l of candida incubated for 4 hours and checked for UV absorbance visually.

Results:

A combination of *Citrullus lanatus, Murraya koenigii and Cinnamomum verum* had a minimal inhibitory concentration of 14 mm against fluconazole (Fig 2). Minimal fungicidal concentration is effective from 10^{-3} where more organisms were killed (Fig 3). Comparison of Zone Of Inhibition (ZOI) results showed that *Citrullus lanatus* had greater zone of inhibition when compared to *Cinnamomum verum, and Murraya koenigii* (Fig 4).

Fig 1-Citrullus lanatus, Cinnamomum verum, and Murraya koenigii- powder form diluted with DMSO against fluconazole (Control group)

Fig 2-Combination of Citrullus lanatus, Cinnamomum verum, and Murraya koenigii powder form against fluconazole. The combined effect is 14mm

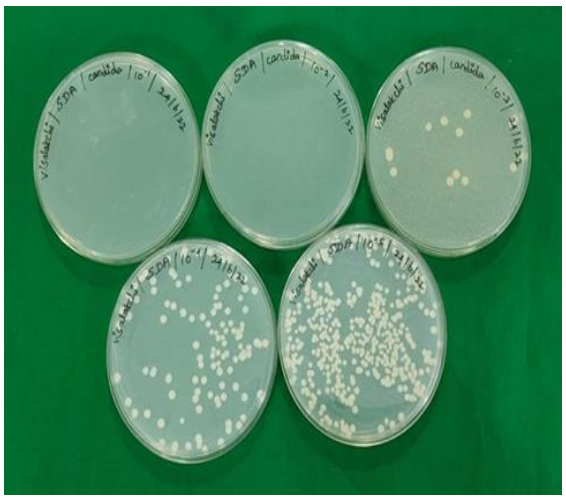


Fig 3-The minimal fungicidal concentration of *Citrullus lanatus, Cinnamomum verum, and Murraya koenigii* powder form against *fluconazole*. From $10^{-}3$ more organisms are killed.

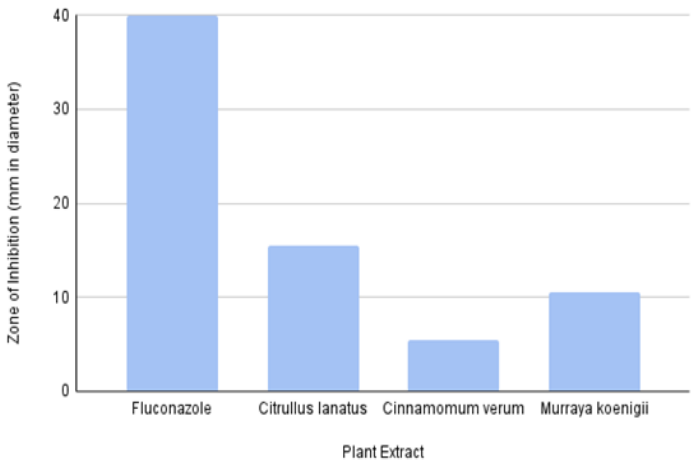


Fig 4-Comparison of Zone Of Inhibition (ZOI) results showed that *Citrullus lanatus* had greater zone of inhibition when compared to *Cinnamomum verum, and Murraya koenigii*

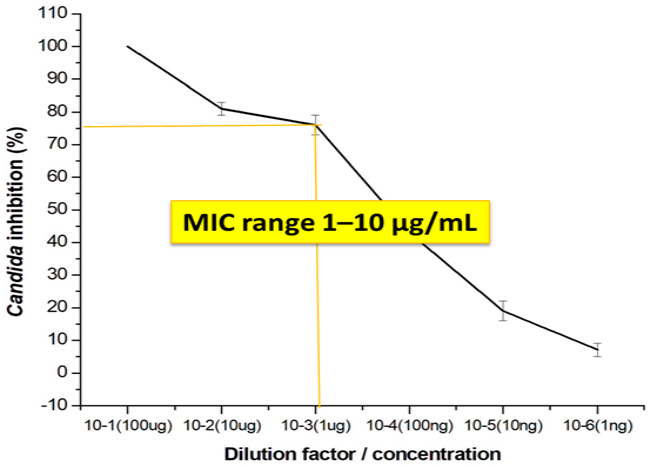


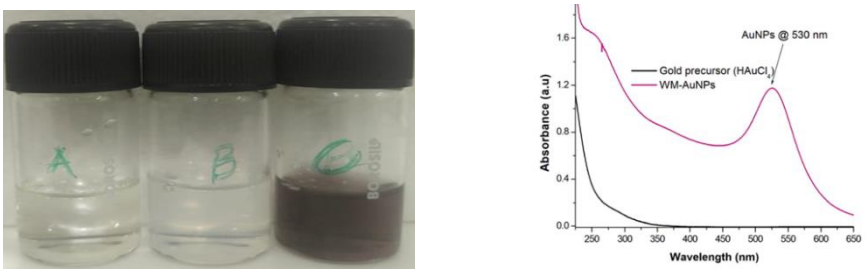
Fig 5-Minimal Inhibitory Concentration (MIC) test- Citrullus lanatus, Cinnamomum verum, and Murraya koenigii

Biosynthesis of gold nanoparticles:

UV spectrum of *Citrullus lanatus* has shown a wavelength of 530nm for gold nanoparticles. SEM images have shown that the particle size was found to be 20-25nm (Fig 7). EDX micrograph shows a peak rise for gold nanoparticles. FTIR reveals the hydroxyl group being associated with gold nanoparticles in *Citrullus lanatus* and has a great effect on *C.albicans* (Fig 8,9) with a minimal inhibitory concentration of 13mm for a concentration of 25 and 19mm for a concentration of 100 and the minimum fungicidal concentration from 10^{-3}.(Fig 10,11,12)

(a) (b) (c)

Fig 6- (a) Citrullus lanatus powder and (b) ethanolic extract Citrullus lanatus and (c) synthesis of (1) gold (Au), (2) Sliver (Ag) and (3) Selenium nanoparticles (SeNPs).

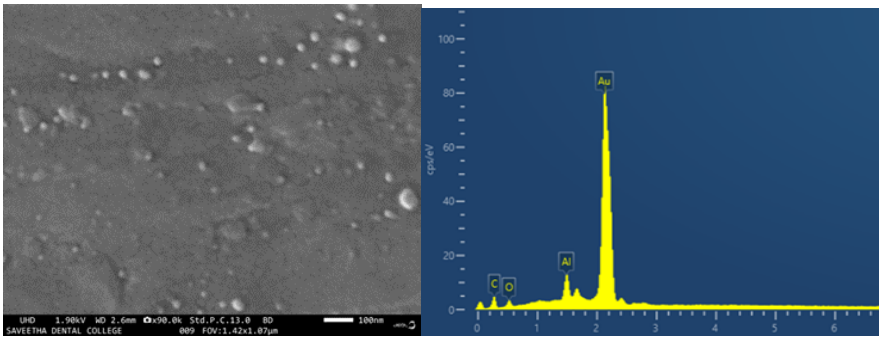


(a) (b)

Fig 7-Biosynthesized *Citrullus lanatus*-AuNPs

(a) Biosynthesis and (b) UV-vis spectrum of *Citrullus lanatus* -AuNPs.

(a) A- *Citrullus lanatus* with silver, B- *Citrullus lanatus* with selenium, and C- *Citrullus lanatus* ethanol extract – 25 µg/mL and gold chloride ($HAuCl_4$ – 125 µM



(a) (b)

Fig 8- (a)SEM image and (b) EDX micrograph of *Citrullus lanatus* -AuNPs

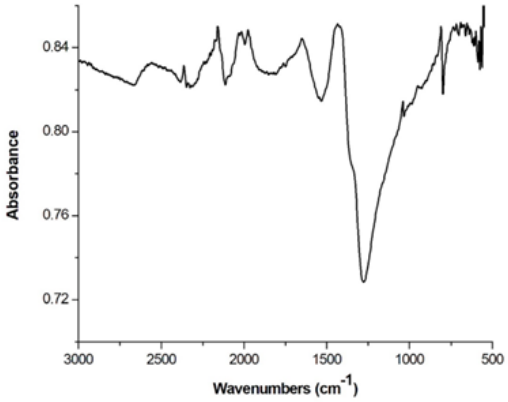


Fig 9-FTIR spectrum of the synthesized *Citrullus lanatus* -AuNPs, associated with the hydroxyl groups (-OH)

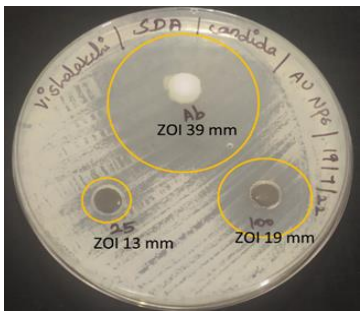


Fig 10-Zone of inhibition (ZOI) measurement. SDA - Sabouraud Dextrose Agar of *Citrullus lanatus*—AuNPs

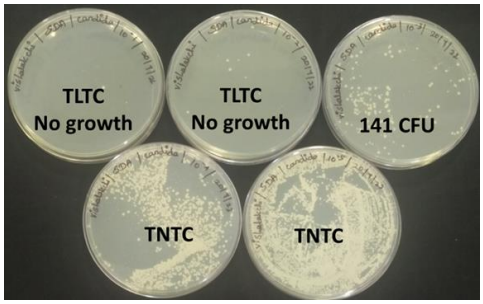


Fig 11-Minimal Fungicidal Concentration (MFC) assessment.

Colony forming unit (CFU). Too Numerous To Count (TNTC). Too Low To Count (TLTC)

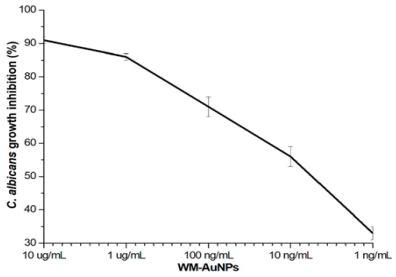


Fig 12-Minimal Inhibitory Concentration (MIC) test-*Citrullus lanatus*-AuNPs

Discussion:

From the results it is evident that *Citrullus lanatus* had greater zone of inhibition when compared to others. The UV spectrum of *Citrullus lanatus* has shown a wavelength of 530nm for gold nanoparticles. *Combination of Citrullus lanatus, Cinnamomum verum, and Murraya koenigii powder form against fluconazole. The combined effect is 14mm.* Higher the concentration greater the zone of inhibition. SEM images have shown that the particle size was found to be 20-25nm. EDX micrograph shows a peak rise for gold nanoparticles. FTIR reveals the hydroxyl group being associated with gold nanoparticles in *Citrullus lanatus* and has a great effect on *C.albicans* with a minimal inhibitory concentration of 13mm for a concentration of 25 and 19mm for a concentration of 100 and the minimum fungicidal concentration from 10^{-3}. The most common fungal infection that affects the oral cavity is candidiasis, which is caused by Candida albicans [11]. All commercially available drugs used for the treatment of candidiasis have adverse effects when used for a long duration. Hence, therapy which has the least side effects is desirable. Plant-based products can be used as an alternative in the treatment of oral candidiasis. *Citrullus lanatus, Murraya koenigii and Cinnamomum verum* were studied in vitro. Plants are a rich source of naturally occurring substances such as flavonoids, tannins, and saponins. They are made from a variety of plant parts, including the flower, leaf, bark, stem, and root sheath. As plants contain various secondary metabolites they can be used for the synthesis with nanoparticles. The application of nanotechnology with plant-based are being extensively studied in recent years [12]. Current research is based on the synthesis of watermelon rind for the management of fungal infections [13]. The effectiveness of different plant extracts and essential oils, including tulsi (O. sanctum), cinnamon (C. verum), garlic (Allium sativum), and lemongrass (Cymbopogon), against Candida albicans was evaluated in a study by Prajapati M. et al. [11]. According to the study, both lemongrass oil and powder had antifungal effects against C. albicans. When lemongrass oil and powder concentrations rise, so does the inhibitory zone. Mahmood observed the antifungal activity of cinnamon powder with higher concentrations [14].

Conclusion:

The present study concludes that the combination of *Citrullus lanatus, Murraya koenigii, and Cinnamomum verum* in powder form, and the biosynthesis of gold nanoparticles with *Citrullus lanatus* has a good effect on *C.albicans.* Greater the zone of inhibition, the higher the concentration. The utilization of *Citrullus lanatus,*

Murraya koenigii, and Cinnamomum verum in suspension or emulsion form as an alternative to synthetic drugs in the management of candidiasis requires further research on a wider scale involving clinical trials.

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