Original article

Synthesizing curcumin-infused hydrogel to inhibit oral fungal formation caused by asthma inhalers

Ha A[1], Loughran S[2]

Submitted: June 14, 2023, Revised: version 1, June 17, 2023, version 2, August 21, 2023, version 3, October 6, 2023
Accepted: October 8, 2023

Abstract
Asthma is an increasingly common chronic condition affecting almost 300 million people worldwide. Corticosteroids, the most effective class of drugs against asthma, are available as inhalers. However, these drugs weaken the mouth and throat's immune systems over time, allowing the fungus *Candida albicans* to proliferate. Oral candidiasis, one of the most common side effect of inhaled corticosteroids, produces painful oral lesions. Although small molecule antifungal agents are an obvious treatment, they too have adverse side effects; such as the fungus developing resistance over time. Curcumin is a naturally ocurring spice with antifungal properties. Its incorporation into a polyvinyl alcohol (PVA) hydrogel was investigated for its ability of inhibit fungal growth. Hydrogels achieve better adherence and higher residence time at the target site, expediting drug delivery. Using the gelation process, the hydrogel was formed using freeze-thaw cycles from an aqueous PVA solution and infused with curcumin or clotrimazole, and coated on filter paper disks. To determine the extent of fungal formation in the petri dishes in the presence of the gel, a zone of inhibition test was performed using the disk diffusion method. It was found that the 3%, 5%, and 7% curcumin treatments significantly inhibited fungal growth compared to the control. The 3% and 7% curcumin-infused PVA hydrogel also significantly inhibited fungal growth compared to clotrimazole.

Keywords
Asthma, Hydrogel, Polyvinyl alcohol, Curcumin, PVA Hydrogel, *Candida albicans*, Oral Candidiasis, Clotrimazole, Antifungal, Bioavailability

[1] Corresponding Author: Abbie Ha, Colleyville Heritage High School, 5401 Heritage Ave, Colleyville, Texas 76034, USA. abbie_ha@yahoo.com
[2] Mentor: Sonya Loughran, Colleyville Heritage High School, 5401 Heritage Ave, Colleyville, Texas 76034, USA. sonya.loughran@gcisd.net

Introduction

As the most common medication for treating asthma, corticosteroid inhalers, which contain steroids that are involved in the immunosuppression of the pulmonary immune system, ineffectively deliver the medication to the lungs (1). Residual drug remains in the mouth and throat, weakening the oral immune system, and allowing *Candida albicans* fungus to proliferate and produce painful oral lesions in a condition called oral candidiasis (1). Oral candidiasis, although not deadly, can cause pain and discomfort, and lead to other conditions such as dysphagia and odynophagia (2, 3). Since current antifungal treatments ineffectively deliver the drug to the target site and few of these treatments exist, in turn enabling their overuse and inducing fungal resistance, curcumin has gained increasing attention in the medical field as an alternative drug to treat oral candidiasis (4, 5).

Curcumin is a natural spice that targets fungal cells by downregulating the production of ergosterol, a sterol that maintains fungal cell wall integrity (6-8). Additionally, curcumin inhibits the production of proteinases and phospholipases, preventing the fungi from adhering to human cells and forming a layer of biofilm (6, 7, 9). However, since curcumin exhibits poor bioavailability, implementing it into a polyvinyl alcohol powder (PVA) hydrogel, a versatile and biocompatible biomaterial, shows promising implications in the biomedical field due to the hydrogel's physiochemical similarity to the native extracellular matrix and its ability to improve the stability of bioactive molecules while maintaining a high concentration of drug at the target site (10-12). Curcumin-infused hydrogels may provide a safer and more effective alternative in treating oral candidiasis when compared to conventional small molecule antifungal treatments while simultaneously addressing the challenges associated with drug delivery and bioavailability.

A hydrogel is a biomaterial with many desirable qualities, such as high porosity, bio-adhesiveness, and a large swelling ratio, making it an ideal material for drug delivery and wound dressing (12, 13). Hydrogels have a soft and rubbery texture that mimics human tissue, maintain a high concentration of drug at the target site over extended periods, retain a moist and protective environment for the wound to heal, and promote the creation of a new extracellular matrix (12, 14). Hydrogels are typically formed using a polymer and a solvent, and they generally need to undergo multiple freeze-thaw cycles to allow the gel to set (15, 16). Polyvinyl alcohol powder was selected as the polymer because it is biocompatible, non-toxic, biodegradable, flexible, and hydrophilic (16). Additionally, a PVA hydrogel can incorporate bioactive molecules within its matrix, such as curcumin, and overcome curcumin's poor bioavailability while enhancing its therapeutic effectiveness (16).

Inspired by the versatility of hydrogels and their potential impact on the field of drug delivery systems, it was hypothesized that the curcumin hydrogels would be more effective in inhibiting *Candida albicans* fungus formation compared to a commonly used antifungal cream containing the antifungal drug

clotrimazole. Accordingly, it was hypothesized that using a PVA-distilled water gel would increase curcumin's bioavailability as well as deliver the drug directly to its target site.

Materials

The materials used were: Family™ Care Clotrimazole as the conventional small molecular comparator, polyvinyl alcohol powder of molecular weight 44.05 KDa (Carolina Biological Supply, NC, USA), curcumin powder (Nova® Nutrition, NJ, USA), potato dextrose agar (item #P0096, Flinn Scientific, IL, USA), *Candida albicans* fungus (ATCCC #10231, Carolina Biological Supply, NC, USA), sterile polystyrene Petri dishes (item #AP8170, 15 x 90 mm diameter, Flinn Scientific, IL, USA), incubator model 10-140 (Grainger®, IL, USA), and 8 mm diameter filter paper disks with medium porosity (Carolina Biological Supply, NC, USA).

Methods

1. PVA Hydrogels

To prepare the PVA hydrogels, 160 mL of distilled water was heated to 35°C using a hot plate. Serving as the control group, a 20% solution of PVA-distilled water was produced with 40 mL of heated distilled water and 8 g of PVA powder. Using the same gel produced for the control, an equal part mixture of hydrogel and the antifungal cream containing clotrimazole, was evenly mixed to serve as the first treatment group. Clotrimazole was mixed into the PVA hydrogel, rather than left separately, to eliminate a potential confounding variable in the experiment, as all the other experimental groups contained a PVA hydrogel. The 3%, 5%, and 7% curcumin concentrations were determined based on a study conducted by Chin et al., in which curcumin concentrations ranging from 2.5%-7.5% had the largest loading capacities in nanoparticles, therefore possessing the greatest drug delivery efficiency (17). Furthermore, Shefa et al. concluded that hydrogels containing more than 7.5% concentration of curcumin released lower amounts of curcumin, while the 5% PVA-curcumin exhibited the largest curcumin release (18). Although the concentration of clotrimazole was reduced from 1% to 0.5% when it was incorporated with the control hydrogel, a study conducted by Pelletier et al. found that there was no significant difference between the minimum inhibitory concentration of clotrimazole concentrations from 0.5 μg/ml to 8 μg/ml on 90% of Candida strains, including *Candida albicans*, concluding that any concentrations within that range would provide similar results (19). Additionally, although the clotrimazole used in this experiment contained other inactive ingredients that could interfere with the fungal growth, Venkatachalam et al. found that a pure sample of clotrimazole had comparable antifungal activity (20). The second treatment group, the 3% curcumin group, was produced with a 20% solution of PVA and 3% curcumin solution, made with 40 mL of heated distilled water, 8 g of PVA powder, and 1.2 g of curcumin. The third treatment group, the 5% curcumin group, was produced with a 20% solution of PVA and 5% curcumin solution, made with 40 mL of heated distilled water, 8 g of PVA powder, and 2 g of curcumin. Lastly, the fourth treatment group, the 7% curcumin group, was produced with a 20% solution of PVA and 7% curcumin

solution, made with 40 mL of heated distilled water, 8 g of PVA powder, and 2.8 g of curcumin. As shown in Figure 1, a magnetic stirrer was used to mix all the solutions until a thick, gel-like consistency was obtained. After preparing all the treatments, the solutions were refrigerated until gelation occurred.

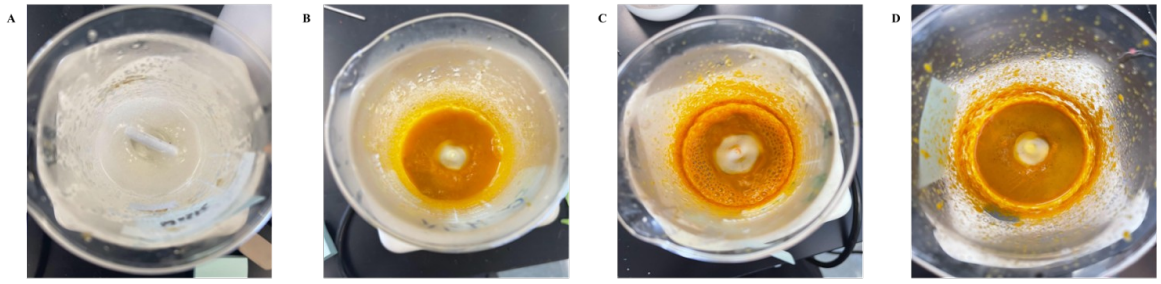

Figure 1. Formulation of curcumin hydrogel treatments. A) Control, B) 3%, C) 5%, D) 7%

2. Petri Dishes

To prepare the Petri dishes, 14.4 g of potato dextrose agar was added to a graduated cylinder with 400 mL of distilled water. The solution was stirred until the solid was evenly distributed. Then, the solution was sterilized by boiling it until it turned transparent. After letting the solution cool for 10 minutes, the agar was poured into sterilized culture dishes, as shown in Figure 2. Wearing gloves and goggles to prevent potential fungal infection and burns from the flame and using an inoculation loop, the *Candida albicans* fungus was streaked using the quadrant streaking technique along each petri dish, leaving three uncultured to act as the negative controls. Working quickly and using the petri dish lid as a shield from contaminants, the petri dishes received as little exposure to the outside environment as possible. The inoculation loop was dipped in ethanol and heated using a Bunsen burner for several seconds before and after each use for additional sterilization and repeated for all petri dishes. The culture was allowed stored for 48 hours in an incubator at 37℃ to cultivate (Figure 3).

3. *Procedure*

After incubating for 48 hours, the 15 Petri dishes with nutrient agar medium and fungus were numbered and randomized into five groups. One group received the PVA hydrogel only, the second group received the PVA hydrogel with 3% curcumin, the third group received the PVA hydrogel with 5% curcumin, the fourth group received the PVA hydrogel with 7% curcumin, and the fifth group received clotrimazole. The petri dishes were randomly assigned to the treatment groups using a random number generator. Using filter paper disks and sterilizing them under a UV light, 12 disks were dipped into each treatment type. Four disks of the same treatment were evenly spaced into each petri dish (Figure 4). Four negative controls were produced by adding all the curcumin filter paper disks to uncultured agar plates and adding no treatment to an

uncultured agar plate, as demonstrated in Figure 5. A positive control was prepared by streaking with Candida (Figure 6). After adding the treatments, the experimental petri dishes were placed in an incubator at 37℃ for 5 days, and after 5 days, the zone of inhibition size was measured, using a meter ruler (d=0.1 cm). The data collected was used to conduct several one-tailed, two-sample t-tests.

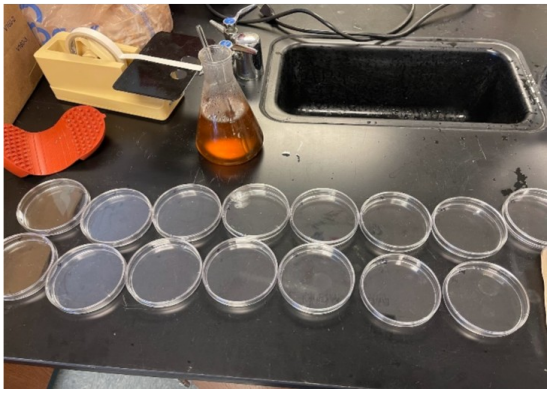

Figure 2. Preparation of petri dishes using potato dextrose agar.

Figure 3. Storage of petri dishes in the incubator

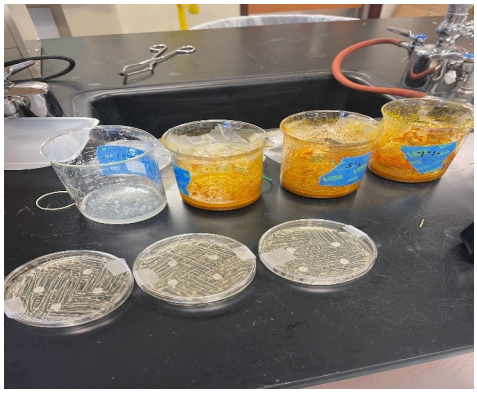

Figure 4. Experimental set-up involving Petri dishes, filter paper disks and hydrogel treatments.

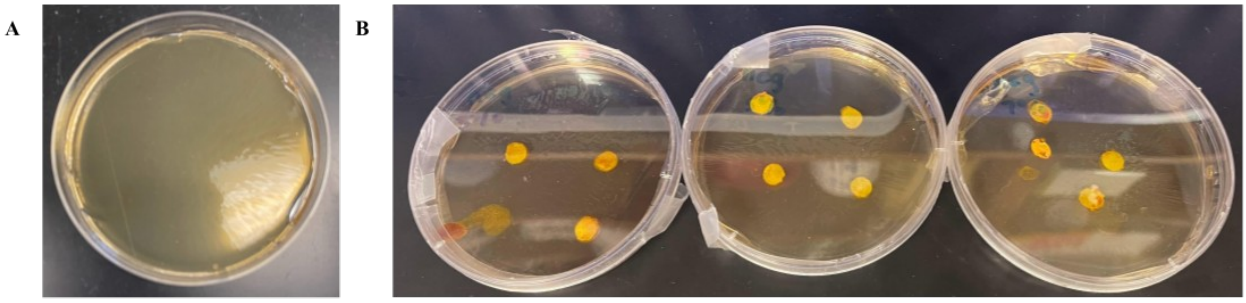

Figure 5. The negative controls after 5 days of incubation. Figure 5a depicts the negative control involving an uncultured plate without clotrimazole or curcumin treatments. Figure 5b depicts the negative controls involving the 3%, 5% and 7% curcumin treatments atop uncultured plates.

Figure 6. Positive control after 5 days of incubation

Results

No growth was observed after 5 days of incubation in any of the negative control petri dishes. In contrast, fungal growth was observed in the positive control after 5 days.

The objective of this experiment was to verify whether a curcumin-infused PVA hydrogel could inhibit fungal growth caused by *Candida albicans* and whether this treatment was more effective than conventional antifungal creams. After 5 days in the incubator, the zones of inhibition, shown in Figure 7, were measured surrounding each filter paper disk in each petri dish. The zones of inhibition of each of the filter paper disks were measured and averaged. These are shown in Table 1.

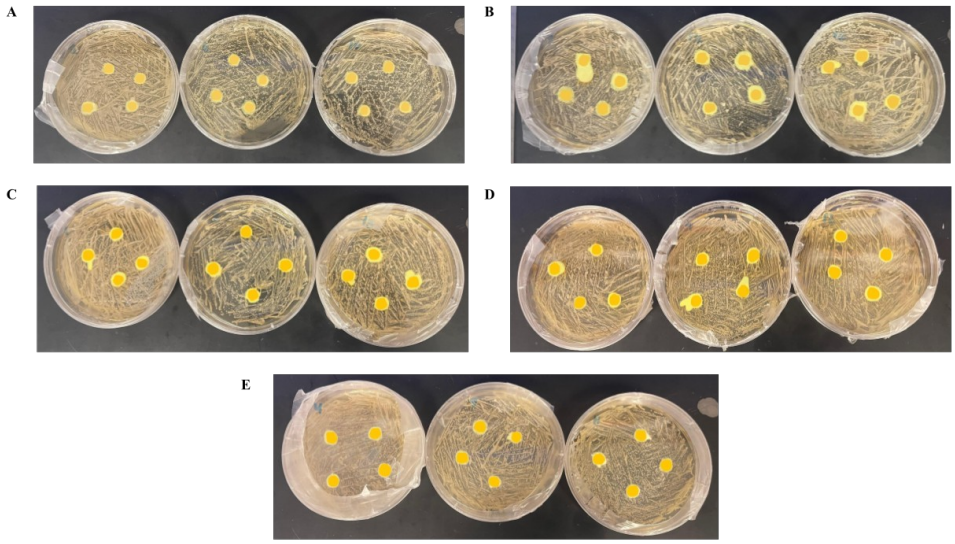

Figure 7. The mean zones of inhibition size for each Petri dishes with curcumin or clotrimazole treatments. A) Control, B) 3% curcumin C) 5% curcumin D) 7% curcumin E) Clotrimazole The orange highlight indicates the location of the filter paper disks and the light-yellow highlight indicates the zone of inhibition.

Using the results from Table 1, several one-tailed two-sample t-tests at an alpha value of 0.05 were conducted to determine whether the results were statistically significant. Based on the results of the experiment, it was concluded that the treatment groups consisting of the 3%, 5%, and 7% curcumin-infused PVA hydrogel were statistically significant in their effect on preventing *Candida albicans* fungal growth compared to the control because the p-values of 0.001, 0.006, and 0.001 respectively, were less than the alpha value of 0.05 (Table 2). Additionally, the 3% curcumin PVA hydrogel and the 7% curcumin PVA hydrogel were statistically significant in their effect on preventing *Candida albicans* fungal growth compared to the clotrimazole because the p-values of 0.007 and 0.002 respectively, were less than the alpha value of 0.05 (Table 2). When comparing Clotrimazole with the 5%

curcumin, the one-tailed significance test showed that the mean zone of inhibition (cm) was not statistically significant between all groups, as the p-value of 0.129 was greater than the alpha value of 0.05. Therefore, all the curcumin-infused PVA hydrogel treatments proved effective against the control, but only the 3% and 7% curcumin-infused PVA hydrogel treatments proved more effective than clotrimazole in inhibiting fungal growth.

Table 1. Zone of inhibition sizes measured after 5 days in the incubator.

Treatment Type	Petri Dish Number	Zone of Inhibition Size (cm)	Average Zone of Inhibition (cm)	Mean Zone of Inhibition (cm)	Standard Deviation (cm)
Control	2	0.8,0.8,0.8,0.8	0.8	0.84	0.079
	6	1.0,0.8,0.8,1.0	0.9		
	10	0.8,0.9,0.8,0.8	0.825		
3% Curcumin	8	1.4,1.0,0.9,1.1	1.1	1.04	0.162
	14	1.0,1.2,0.9,1.2	1.075		
	15	0.9,0.9,1.1,0.9	0.95		
5% Curcumin	1	0.9,0.9,1.0,0.9	0.925	0.94	0.100
	3	0.8,1.1,1.0,0.8	0.925		
	13	1.1,1.0,0.9,0.9	0.975		
7% Curcumin	5	1.0,1.1,1.2,0.9	1.05	1.02	0.103
	7	1.1,1.0,1.1,0.9	1.025		
	12	1.0,1.1,0.9,0.9	0.975		
Clotrimazole	4	0.9,1.0,0.8,0.9	0.9	0.90	0.074
	9	1.0,0.9,0.9,0.8	0.9		
	11	0.9,1.0,0.9,0.8	0.9		

Table 2. The calculated p-values after conducting one-tailed two-sample t-tests. The green highlight indicates p-values less than the alpha value of 0.05, indicating a statistically significant difference. A (-) indicates that a p-value is not applicable.

Treatment Type	vs. Control	vs. 3% Curcumin	vs. 5% Curcumin	vs. 7% Curcumin	vs. clotrimazole
Control	-	< 0.001	0.006	< 0.001	0.038
3% Curcumin	0.038	0.007	0.129	0.002	-

Discussion

This objective of this study was to determine whether curcumin would be an effective drug in inhibiting *Candida albicans* formation and whether a PVA hydrogel would enhance drug delivery to the target site and serve as a better alternative to current antifungal drugs. While the data supported the notion that the curcumin hydrogels were indeed effective and that they were more effective than traditional treatments, only the 5% curcumin treatment did not prove to be more effective than clotrimazole in inhibiting fungal formation.

Although most of the curcumin treatments showed promise in improving the current antifungal treatment, it is important to note that the concentrations of the curcumin were higher than the minimum inhibitory concentration (MICs). For instance, the curcumin concentrations of 3%, 5%, and 7% would be more than six, ten, and fourteen, respectively, times greater than the MIC of 5000 μg/mL for curcumin (21). Additionally, the MIC of the clotrimazole treatment was over sixteen times greater than its MIC of 0.62 μg/mL (22). Therefore, it would be inaccurate to conclude that either clotrimazole or curcumin was more effective than the other since the treatments were never diluted to equivalent MICs. However, this study aimed to determine if curcumin was effective at varying full concentration levels, and whether curcumin could be more effective than conventional treatments, as this may reveal much promise in the field of nanomaterials and curcumin's role in drug delivery. This experiment also did not test curcumin in the absence of PVA, hence any inferences as to the sustained delivery at the site of infection and increase in bioavailability are conjectural and derived from literature data.

This research could be applied to the field of antifungal treatments, which are often overused or misused, hence often causing increased fungal resistance. The use of curcumin as a topical antifungal agent may effectively treat oral candidiasis and potentially many other fungal conditions (23). Many of the challenges with current formulations are that they do not deliver the drug effectively to the target site and that the pathogen develops resistance, as in the case of *Candida albicans* (23). Curcumin is effective in a wide range of applications, such as anti-cancer, anti-bacterial, and antifungal (24). Curcumin is also antimutagenic hence it can potentially impede pathogen resistance (25). Furthermore, since PVA hydrogels have an easily tunable matrix that allows incorporation of a variety of drugs, PVA hydrogels could be used to administer other non or sub-biocompatible agents, which otherwise could not be so administered (26). The hydrogels can also take on various structures and release drug at differing rates as needed (27). The future of PVA hydrogels appears bright as they may fulfill the increased need for alternative methods for drug delivery and wound dressing.

Limitations and future directions

Only one concentration of clotrimazole was used, and like the curcumin concentrations, perhaps different concentrations could have had varying therapeutic effectiveness, making the zones of inhibition values lower than they should be. In the future, to reduce the

variability in the zone of inhibition sizes, obtaining an autoclave to sterilize the agar would further prevent contamination, and sealing the petri dishes using Parafilm® would prevent moisture loss. Additionally, a larger range of curcumin concentrations could be tested to determine the optimal concentration, while also applying the curcumin-infused PVA hydrogel to a greater variety of *Candida* variants.

Conclusion

On average, the 3% curcumin hydrogel produced the largest zone of inhibition (1.04 cm) compared to the rest of the curcumin treatments and clotrimazole treatment. However, the 3% curcumin group also exhibited the largest standard deviation, hence a larger study with more samples and more statistical power is necessary to determine efficacy. Curcumin's antifungal nature can mostly be attributed to its antioxidant properties (a property clotrimazole lacks), in which free radicals are reduced, mitigating their damaging effects (28). Instead, clotrimazole impairs the permeability of the fungal cell wall, but oftentimes clotrimazole is less efficacious because the strong fungal cell wall resists disruption (29). Accordingly, the one-tailed, two-sample t-tests concluded that all the zones of inhibition sizes for the curcumin treatments, excluding the 5% curcumin, were significantly greater than that of clotrimazole. Although the concentrations for each treatment were not conducted at equivalent MICs, at the concentrations conducted in this study, the fungus was susceptible to all. However, it is important to understand the adverse effects of each treatment at the levels used in this experiment, as clotrimazole may cause itching, vomiting, and nausea at 10 mg dosages (0.2% cream), and curcumin may cause diarrhea and nausea at 0.5 g-1.2 g dosages (29, 30). To address these issues, if the treatment is diluted to the MICs as mentioned previously, the adverse effects would decrease as the concentrations of the drugs decreased.

Acknowledgments

I would like to recognize Mrs. Loughran, my Chemistry Research and Design teacher, for guiding me throughout this entire research process and being my biggest inspiration. I would also like to recognize Dr. Popham and Mrs. Burnett for supporting and advising my research.

References

1. Srakocic, S. (2021). *Oral thrush and inhalers: Causes, symptoms, and treatment*. Healthline. https://www.healthline.com/health/dental-and-oral-health/thrush-from-inhaler

2. Narayanan, V. A., Rao, B. P. (2014). Buccal In Situ Gel Containing Curcumin for the Treatment of Oral Thrush. *World Journal of Pharmacy and Pharmaceutical Sciences*, *3*(11), 633–645.

3. Ruhnke, M. (2014). Antifungal stewardship in invasive candida infections. *Clinical Microbiology and Infection*, *20*, 11–18. https://doi.org/10.1111/1469-0691.12622

4. Daliri, F., Azizi, A., Goudarzi, M., Lawaf, S., Rahimi, A. (2019). In vitro comparison of the effect of photodynamic therapy with curcumin and methylene blue on candida albicans colonies. *Photodiagnosis and Photodynamic Therapy*, *26*, 193–198. https://doi.org/10.1016/j.pdpdt.2019.03.017

5. Rabaan, A. A., Sulaiman, T., Al-Ahmed, S. H., Buhaliqah, Z. A., Buhaliqah, A. A., AlYuosof, B., et al. (2023). Potential strategies to control the risk of antifungal resistance in humans: A comprehensive review. *Antibiotics*, *12*(3), 608. https://doi.org/10.3390/antibiotics12030608

6. Chen, E., Benso, B., Seleem, D., Ferreira, L. E., Pasetto, S., Pardi, V., Murata, R. M. (2018). Fungal-host interaction: Curcumin modulates proteolytic enzyme activity of candida *albicans and* inflammatory host response in *vitro*. *International Journal of Dentistry*, *2018*, 1–7. https://doi.org/10.1155/2018/2393146

7. Cheraghipour, K., Ezatpour, B., Masoori, L., Marzban, A., Sepahvand, A., Rouzbahani, A. K., et al. (2021). Anti-candida activity of Curcumin: A systematic review. *Current Drug Discovery Technologies*, *18*(3), 379–390. https://doi.org/10.2174/1570163817666200518074629

8. Kumar, A., Dhamgaye, S., Maurya, I. K., Singh, A., Sharma, M., Prasad, R. (2014). Curcumin targets cell wall integrity via calcineurin-mediated signaling in candida albicans. *Antimicrobial Agents and Chemotherapy*, *58*(1), 167–175. https://doi.org/10.1128/aac.01385-13

9. Bisso, B. N., Makuété, A. L., Tsopmene, J. U., Dzoyem, J. P. (2023). Biofilm formation and phospholipase and proteinase production in Cryptococcus neoformans clinical isolates and susceptibility towards some bioactive natural products. *The Scientific World Journal*, *2023*, 1–7. https://doi.org/10.1155/2023/6080489

10. Anand, P., Kunnumakkara, A. B., Newman, R. A., Aggarwal, B. B. (2007). Bioavailability of curcumin: Problems and promises. *Molecular Pharmaceutics*, *4*(6), 807–818. https://doi.org/10.1021/mp700113r

11. Zhu, J., Marchant, R. E. (2011). Design properties of hydrogel tissue-engineering scaffolds. *Expert Review of Medical Devices*, *8*(5), 607–626. https://doi.org/10.1586/erd.11.27

12. Vigata, M., Meinert, C., Hutmacher, D. W., Bock, N. (2020). Hydrogels as drug delivery systems: A review of current characterization and evaluation techniques. *Pharmaceutics*, *12*(12), 1188. https://doi.org/10.3390/pharmaceutics12121188

13. Fu, J., in het Panhuis, M. (2019). Hydrogel Properties and Applications. *Journal of Materials Chemistry B*, *7*(10), 1523–1525. https://doi.org/10.1039/c9tb90023c

14. Kouchak, M. (2014). In situ gelling systems for drug delivery. *Jundishapur Journal of Natural Pharmaceutical Products*, *9*(3). https://doi.org/10.17795/jjnpp-20126

15. Butylina, S., Geng, S., Oksman, K. (2016). Properties of AS-prepared and freeze-dried hydrogels made from poly(vinyl alcohol) and cellulose nanocrystals using freeze-thaw technique. *European Polymer Journal*, *81*, 386–396. https://doi.org/10.1016/j.eurpolymj.2016.06.028

16. Muller, R., Innerebner, F. (2005). *EP1781251A2 - polyvinyl alcohol gels, especially in situ gelling gels*. Google Patents. https://patents.google.com/patent/EP1781251A2/en

17. Chin, S. F., Mohd Yazid, S. N., Pang, S. C. (2014). Preparation and characterization of starch nanoparticles for controlled release of Curcumin. *International Journal of Polymer Science*, *2014*, 1–8. https://doi.org/10.1155/2014/340121

18. Shefa, A. A., Sultana, T., Park, M. K., Lee, S. Y., Gwon, J.-G., Lee, B.-T. (2020). Curcumin incorporation into an oxidized cellulose nanofiber-polyvinyl alcohol hydrogel system promotes wound healing. *Materials & Design*, *186*, 108313. https://doi.org/10.1016/j.matdes.2019.108313

19. Pelletier, R., Peter, J., Antin, C., Gonzalez, C., Wood, L., Walsh, T. J. (2000). Emergence of resistance of *candida albicans* to clotrimazole in human immunodeficiency virus-infected children: In vitro and clinical correlations. *Journal of Clinical Microbiology*, *38*(4), 1563–1568. https://doi.org/10.1128/jcm.38.4.1563-1568.2000

20. Venkatachalam, S., Harsha, M., Pooja, M., Paranjothy, M., Kumar, R. (2017). Enhanced bio-adhesion and sustained delivery of clotrimazole encapsulated solid lipid nanoparticles loaded in hyaluronic acid gel as anti- fungal therapy. *British Journal of Pharmaceutical Research*, *17*(1), 1–12. https://doi.org/10.9734/bjpr/2017/34063

21. Adamczak, A., Ożarowski, M., Karpiński, T. M. (2020). Curcumin, a natural antimicrobial agent with strain-specific activity. *Pharmaceuticals*, *13*(7), 153. https://doi.org/10.3390/ph13070153

22. Meneses, M. L., Recalde, M., Martin, P. L., Pardo, A. G. (2022). Antifungal activity of silver nanoparticles and clotrimazole against candida spp.. *Brazilian Journal of Pharmaceutical Sciences*, *58*. https://doi.org/10.1590/s2175-97902022e18719

23. Sousa, F., Ferreira, D., Reis, S., Costa, P. (2020). Current insights on antifungal therapy: Novel nanotechnology approaches for Drug Delivery Systems and new drugs from natural sources. *Pharmaceuticals*, *13*(9), 248. https://doi.org/10.3390/ph13090248

24. Xu, X.-Y., Meng, X., Li, S., Gan, R.-Y., Li, Y., Li, H.-B. (2018). Bioactivity, health benefits, and related molecular mechanisms of curcumin: Current progress, challenges, and Perspectives. *Nutrients*, *10*(10), 1553. https://doi.org/10.3390/nu10101553

25. Shukla, Y., Arora, A., Taneja, P. (2002). Antimutagenic potential of curcumin on chromosomal aberrations in wistar rats. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *515*(1–2), 197–202. https://doi.org/10.1016/s1383-5718(02)00016-5

26. Tse, J. R., Engler, A. J. (2010). Preparation of hydrogel substrates with tunable mechanical properties. *Current Protocols in Cell Biology*, *47*(1). https://doi.org/10.1002/0471143030.cb1016s47

27. Kim, S. W., Okano, T. (1992). Hydrogels: Swelling, Drug Loading, and Release. *Pharmaceutical Research*, *9*, 283–290. https://doi.org/https://doi.org/10.1023/A:1015887213431

28. Stringaro, A., Colone, M., Angiolella, L. (2018). Antioxidant, antifungal, antibiofilm, and cytotoxic activities of Mentha spp.. essential oils. *Medicines*, *5*(4), 112. https://doi.org/10.3390/medicines5040112

29. Khatter, N. J., Khan, M. A. (2023). *Clotrimazole*. National Library of Medicine. https://www.ncbi.nlm.nih.gov/books/NBK560643/

30. Hewlings, S., Kalman, D. (2017). Curcumin: A review of its effects on human health. *Foods*, *6*(10), 92. https://doi.org/10.3390/foods6100092