



Assessment Swot on Performance of Micronized Itraconazole against *Candida albicans* and *Aspergillus niger* for Superior Health Security

N. V. S. Venugopal^{1*} and K. Bujji Reddy¹

¹Department of Chemistry, Institute of science, GITAM (Deemed to be niversity), Visakhapatnam A.P, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i49B33368

Editor(s):

- (1) Aurora Martinez Romero, Juarez University, Mexico.
(2) Dharmesh Chandra Sharma, G. R. Medical College & J. A. Hospital, India.
(3) Paola Angelini, University of Perugia, Italy.

Reviewers:

- (1) Luay Thanoon Younis, Universiti Teknologi MARA, Malaysia.
(2) A. Barghouthi, Al-Quds University, Palestine.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/76665>

Original Research Article

Received 05 August 2021
Accepted 10 November 2021
Published 13 November 2021

ABSTRACT

Background: Antifungal medication is used in adults to indulge infections caused by fungi. This includes infections in any part of the body including the lungs, mouth or throat, toenails, or fingernails. The widespread fungal pathogens causing stern insidious infections surrounded by immuno compromised patients are *Candida albicans* and *Aspergillus niger*. Itraconazole, is an antifungal medication used to treat a number of fungal infections. Itraconazole undergoes rapid metabolism to form hydroxyitraconazole, which also contributes to the anti-fungal activity exhibited by the parent compound. In the present study the assessment of plain Itraconazole and micronized Itraconazole material for increasing health safety and its biological evaluation by using *Candida albicans* and *Aspergillus niger* was reported.

Methods: The determination of particle size of the micronized material which is a very fine powder and its characteristics was carried out by Dynamic light scattering(DLS) and Scanning Electron Microscopy(SEM).Structural identification was done by UV-spectroscopy and Fourier transform Infrared spectroscopy(FT-IR).

Results: An active pharma ingredient having antifungal activity was selected and micronized using Microniser to reduce the particle size as small as possible. The product antifungal activity was reviewed between untreated material (large particle size, 200 microns) and reduced particle size (micro particles, 2.72 microns). Size reduction showed an increased efficiency of antifungal activity

Conclusion: The antifungal activity of Itraconazole can be enhanced if the coarse large particles are micronized to significantly (>70 folds) smaller particles.

Keywords: Itraconazole ; Micronization; Electron microscopy; Bioassay.

1. INTRODUCTION

A new triazole antifungal agent is Itraconazole. Itraconazole (ITZ) ($C_{35}H_{38}Cl_2N_8O_4$) is an antifungal medication used to treat a number of fungal infections. Itraconazole is a relatively well-tolerated drug (although not as well tolerated as fluconazole or voriconazole) and the range of adverse effects it produces is similar to the otherazole antifungals.

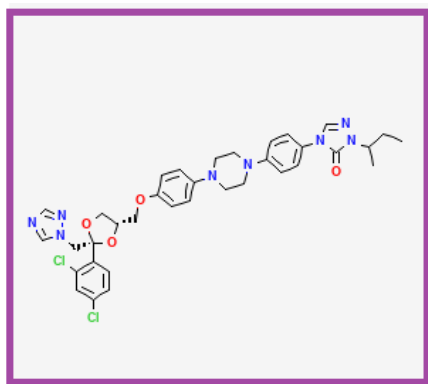


Fig. 1. Structure of ITZ

Many types of fungi live harmlessly on our skin and in other places in the environment. However, some types of fungi can thrive and multiply on the surface of our bodies to cause infections of the skin, mouth or vagina. The most common fungi to cause skin infections are the tinea group of fungi. A common infection of the mouth and vagina is called thrush. It is caused by an overgrowth of a yeast (which is a type of fungus) called candida. Fungal infections sometimes occur within the body as well. You are more at risk of developing an internal fungal infection if your immune system does not work properly. For example, if you are having chemotherapy, or if you are taking medicines for rheumatic disease, or if you have HIV/AIDS. Internal fungal infections can be serious. Itraconazole is used to treat fungal infections. It works by killing yeast and fungi.

Ming Yao [1] studied the bioanalytical methods available for the quantitation of both itraconazole and hydroxyitraconazole. M.P. Ducharme et al. observed that the serum concentrations of Itraconazole and hydroxyitraconazole serum concentrations are reduced more than tenfold by phenytoin [2] (an anti-epileptic drug, also called an anticonvulsant). Mouton placed Pharmacokinetics of itraconazole and hydroxyitraconazole in healthy subjects after single and multiple doses of a novel formulation [3]. Increased oral bioavailability of itraconazole and its active metabolite, 7-hydroxyitraconazole, when coadministered with a vitamin C beverage in healthy participants were studied [4]. Sebastian described that Itraconazole-remdesivir and fluoxetine-remdesivir combinations are promising starting points for therapeutic options to control SARS-CoV-2 infection and severe progression of COVID-19 [5]. Clinical pharmacokinetic monitoring of itraconazole is warranted in only a subset of patients [6]. ITZ offers greater benefit than conventional therapies (griseofulvin and ketoconazole) in terms of efficacy and tolerability, wider clinical experience is required to determine its merits relative to the newer agents, terbinafine and fluconazole [7]. At present time Because of the high mortality of invasive fungal infections (IFIs), appropriate exposure to antifungals appears to be crucial for therapeutic efficacy and safety [8]. A simple, rapid ultra-performance liquid chromatography (UPLC) method was developed for the analysis of itraconazole and its associated production impurities were reported [9]. Fungal prophylaxis has been proven effective in certain high menace patients such as bone marrow transplant and other immune concession patients. Bioassays afford several practical advantages, such as simplicity, rapidity and economy in disease and patient management. Several studies describe ITZ bioassay using *Candida* genus [10-14]. This study aimed at to evaluate the performance of micronized ITZ to increase health safety and its bioassay by using *Candida albicans* and *Aspergillus niger*.

2. MATERIALS AND METHODS

2.1 Materials

Itraconazole (ITZ; $C_{35}H_{38}Cl_2N_8O_4$) working standard was procured from Granules India limited, Visakhapatnam, AP, India. All other chemicals used were of analytical reagent grade.

2.2 Instrumentation

A laser diffraction technique from Malvern 3000 was used to analyse the particle size of the all the batches. Fourier Transform Infra red Spectroscopy (FTIR) (Bruker) was used to characterize and identify the organic molecule. Surface topology was determined by using scanning electron microscopy (SEM) (ZEISS EVO 18).

2.3 Preparation of Untreated Material

The white crystalline hygroscopic technical active pharmaceutical ingredient sample was firstly analysed for identification of impurities. The impurities present in the sample are about 0.06%. The residual solvents like methanol, acetone, ethyl acetate, toluene etc were not detected. The bulk density of the ITZ was 0.63g/mL (untapped) and tapped 0.76g/mL. The sieve analysis was conducted and ITZ was passed through 20 mesh. The loss on drying was 0.22% w/w.

2.4 Preparation of Micronized Material

The plain material was transferred into a dry Micronizer equipment with pressure controllers of feeding pressure and micronization pressure. The sample was poured into micronizer hopper with a feed rate of 4 Kg air pressure and 6 Kg micronization pressure with the help of moisture free air. The same procedure was repeated for three cycles to get maximum micronized material.

2.5 Test Microorganism and Continuance

The yeast and fungi strains of *Candida albicans* and *Aspergillus niger* were used and have been obtained from GVK Bio.Sci, Hyderabad, India. The pathogenic strains under test were maintained on nutrient agar slants and stored at 4°C. The slants were preserved in 25 % glycerol for long period storage at -80°C.

2.6 Antifungal Activity Preparation (Agarwell Diffusion)

In antifungal activity, the potato dextrose agar medium was suspended in 100mL distilled water and heated till complete dissolution. The medium and glass petri-dishes were autoclaved at a pressure of 15 Pascal (Pa) for 20 min. The medium was cooled to 50°C and poured in to sterile petri-dishes under aseptic conditions in a laminar flow chamber.

3. RESULTS AND DISCUSSION

The smaller and finer the particles, the weaker the barriers to dissolution and it leads to higher dissolution rate resulting from a substantial increase of surface area to volume ratio. The other benefits of particle size reduction was increased conductivity, smooth surface quality and high quality product function. The surface modification of ITZ particles showed remarkable enhancement of antifungal activity.

3.1 DLS Analysis

The diameter of the micronized ITZ was obtained by pade laplace dispersion. It mainly depends on Rayleigh scattering, oscillations, Brownian movement and fluorescence exponential decay. In this method a time dependent signal was transformed into the hydro colloidal solution and it results in the exponential decay of the particles. The transformed laplace signal is defined as an infinite integral over time. Through this transformed signal number of components in the micronized ITZ solution can be easily obtained. It can be clearly depicted in the following pade laplace dispersion graph shown in Fig. 2. 1 g of sample is loaded into the sample holder and analysed using laser diffraction particle size analyser using dry accessory. One milli litre of micronized ITZ solution was suspended in 5ml of water. The resultant hydro dispersed suspension was analyzed with DLS at 25-40°C. The average particle size distribution (shown in Figs 2 and 3) was appeared 2.72µm.

3.2 Fourier-transform Infra Red Spectroscopy (FT-IR) Analysis

FT-IR analysis would help to identify the functional groups by placing a small amount of untreated and micronized ITZ compound in potassium bromide (KBr) pellets in transmittance mode. FT-IR analyses were carried out to identify

the possible if any change in the functional groups. Fig. 4 shows the chemical stability of the micronized form as indicated by the recorded spectra in the range of 400–4000cm⁻¹.The FTIR studies (shown in Fig. 4) reveal that there was no change in the chemical structure of plain and as well as micronized ITZ as evident from the presence of peaks due to the same functional group.

3.3 UV-Visible Spectral Study

To confirm the formation and the stability of micronized ITZ material, UV-Vis spectra is

necessary. The maximum absorption spectra of plain and micronized form of ITZ was observed at 262nm.(shown in Figs.5and 6).

3.4 SEM Analysis

The surface topology and size of the ITZ material were obtained by SEM at (35–45.00 k_v) magnification, 15 kv vacuum. A drop of encapsulation complex is taken on the stub and it was air dried then subjected to sputtering by using sputter coater. The SEM image shown in Fig. 7 reveals the homogeneity in shape and it is regularity among different scan regions.

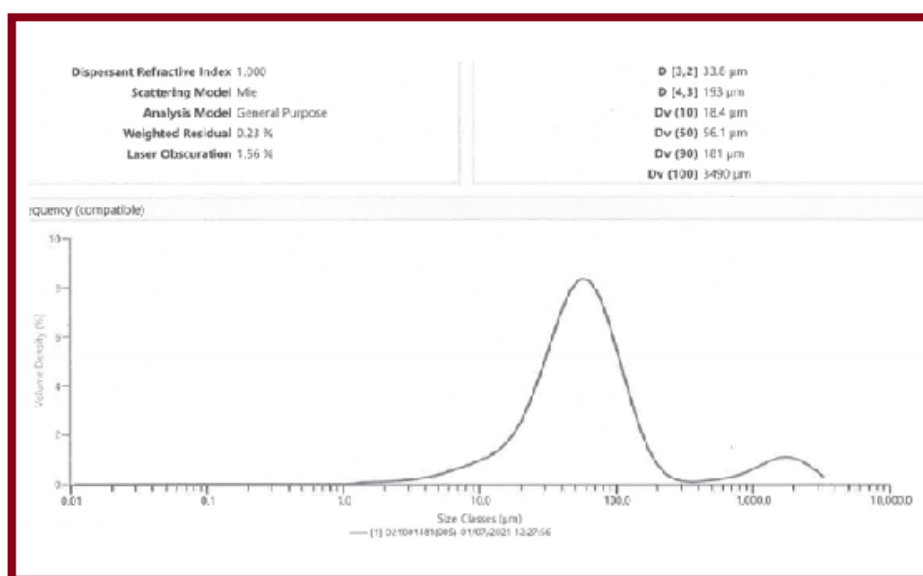


Fig. 2. Size distribution of plain ITZ material

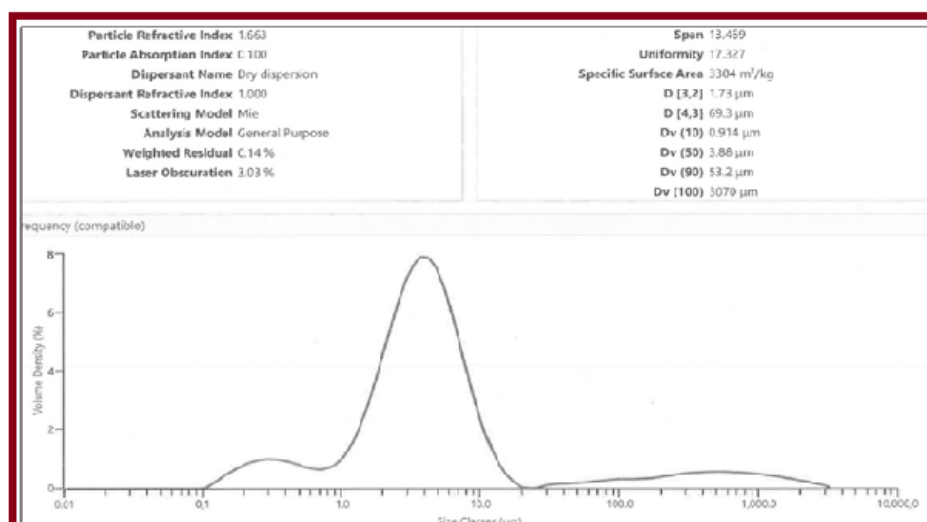


Fig. 3. Size distribution of micronized ITZ material

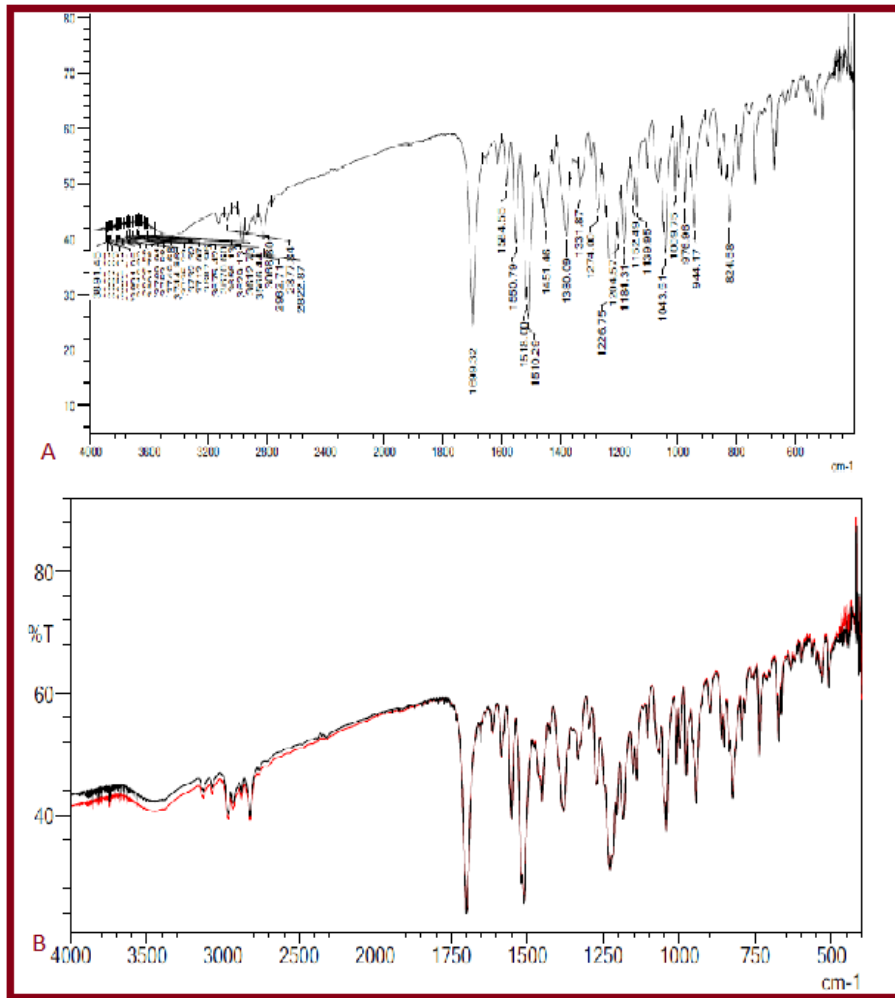


Fig. 4. IR spectra: A:Standard spectrum B: Comparison spectrum (overlay) of plain and micronized showing identical profiles

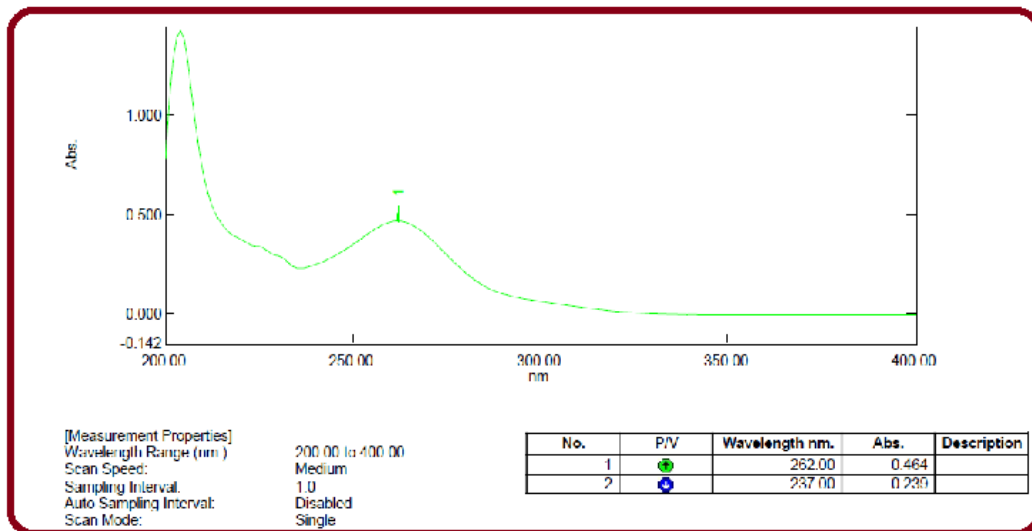


Fig. 5. UV spectra of plain ITZ material

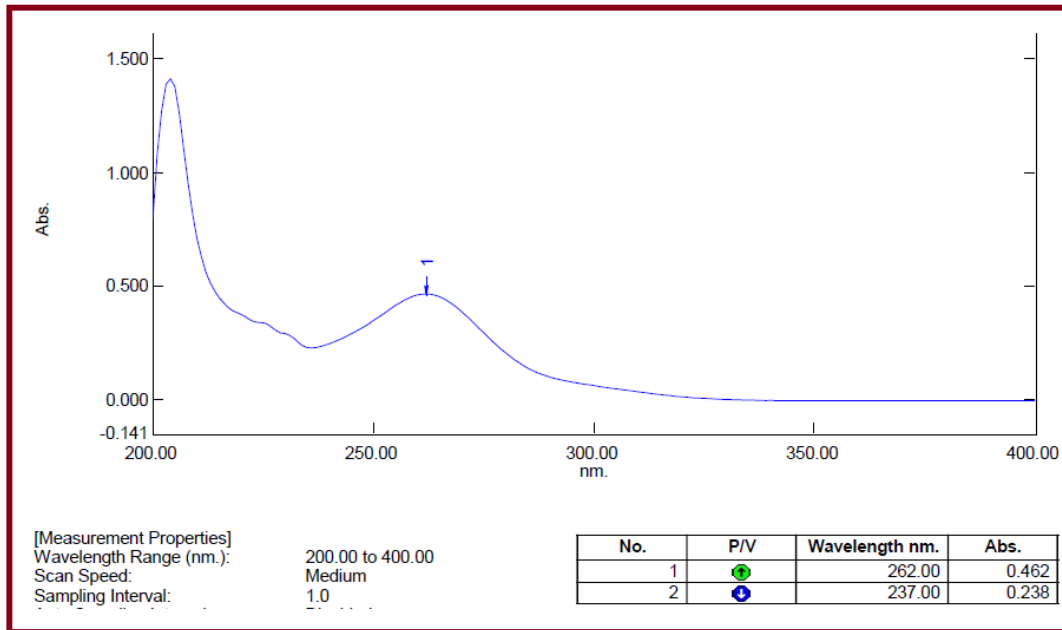


Fig. 6. UV spectra of micronized ITZ material

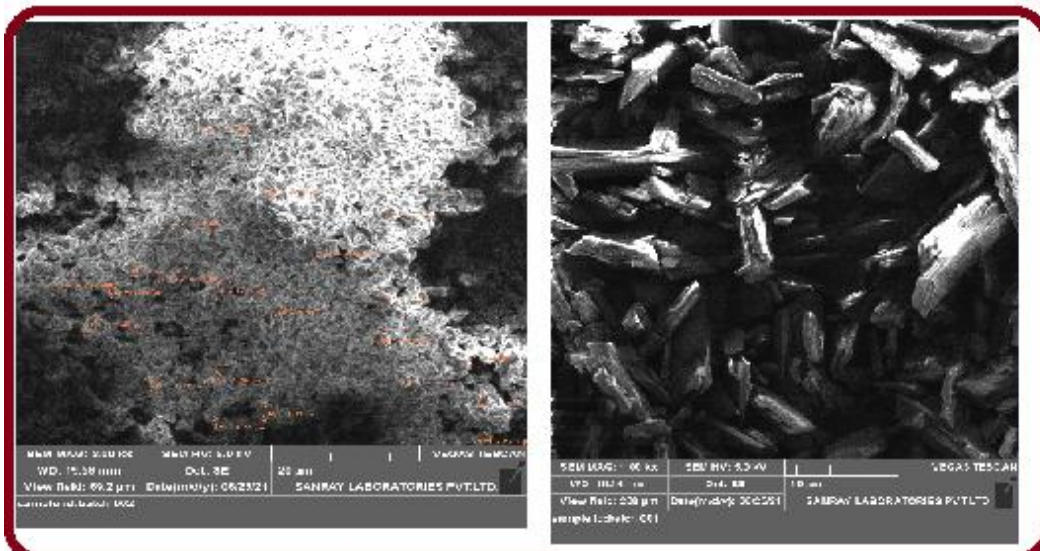


Fig. 7. SEM images of Micronized ITZ material

3.5 Application of Micronized ITZ on *Candida albicans* and *Aspergillus Niger* Agar Diffusion Method

Inoculated a previously Liquefied medium appropriate to the assay with the requisite quantity of suspension of the micro-organism *Candida albicans* and *Aspergillus niger*. Added the suspension to the media (sabouraud Dextrose Agar and Soyabean casein digest agar

) and immediately pour the inoculated media into the Petri dishes (or) large rectangular plates to give a depth of 3 to 5mm. Ensured that the layers of medium are uniform in thickness, by placing the dishes or plates on a level surface. Stored the prepared plates in a manner so as to ensure that no significant growth or death of the test organism occurs before the plates are used and that the surfaces of the agar layer dry at the time of use. known concentration of the sample that is

10mg/mL of Itraconazole(ITZ) is prepared 3 to 5 mm holes are made on the separate agar plates for two batches that is 001(normal) and 002 (Micronized) for both the above mentioned organisms with the help of sterile borer.Known concentration of both the products that was 1.0,2.0 and 10mg/mL is added in to the holes for respective organisms. The volume of solution added to each cylinder must be uniform Left the

plates standing for 1 to 4hrs at room temperature under Laminar air flow Incubated them for about 3 to 5 days at 20 to 25^oc. Accurately measured the diameters or areas of the circular inhibition zones.The results obtained about the effectiveness of ITZ on *Candida albicans* and *Aspergillus niger* were given in Table 1. The anti-fungal activity of plain and micronized ITZ was shown in Fig. 8.

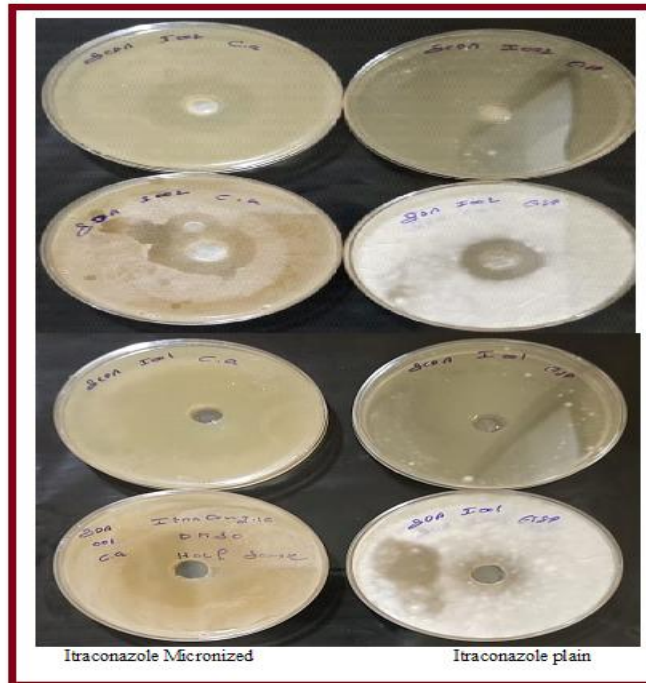


Fig. 8. Antifungal activity of plain and micronized ITZ material

Table 1. The effectiveness of ITZ on *Candida albicans* and *Aspergillus niger*

Organism	Zone of Inhibition for Itraconazole (Micronized) sabouraud Dextrose Agar			Zone of Inhibition for Itraconazole (Normal) sabouraud Dex trose Agar		
	Plate -1	Plate-2	Avg	Plate -1	Plate-2	Avg
<i>Aspergillus niger</i>	0.9 cm	0.7 cm	0.8 cm	0.6 cm	0.4 cm	0.5 cm
<i>Candida albicans</i>	1.3 cm	1.2 cm	1.25 cm	0.8 cm	0.8 cm	0.8 cm
Organism	Zone of Inhibition for Itraconazole (Micronized) Soyabean casein digest agar			Zone of Inhibition for Itraconazole (Normal) Soyabean casein digest agar		
	Plate -1	Plate-2	Avg	Plate -1	Plate-2	Avg
<i>Aspergillus niger</i>	1.3 cm	1.5 cm	1.4 cm	0.9 cm	1.0 cm	0.95 cm
<i>Candida albicans</i>	1.9 cm	1.6 cm	1.75 cm	1.0 cm	1.1 cm	1.1 cm

4. CONCLUSION

Aspergillus fumigatus and *C albicans* are the two most common fungal pathogens causing severe invasive infections among immuno compromised patients. ITZ is a -triazole antifungal agent. In the mechanism of ITZ involves the interruption of the conversion of lanosterol to ergosterol via binding to fungal cytochrome P-450 and succeeding distraction of fungal membranes. From the above study, it is proved that the efficiency of ITZ in terms of antifungal activity of micronized material shows increased efficiency when compared to ITZ plain material (coarser type particles).

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

We are thankful to the management, faculty of chemistry, GITAM University Visakhapatnam, Andhra Pradesh, India for their support and encouragement given to us.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yao M, Srinivas NR. Bioanalytical methods for the determination of itraconazole and hydroxyitraconazole: overview from clinical pharmacology, pharmacokinetic, pharmacodynamic and metabolism

- perspectives. Biomed Chromatogr. 2009; 23(7):677-91.
DOI: 10.1002/bmc.1186. PMID: 19309762.
2. Ducharme MP, Slaughter RL, Warbasse LH, Chandrasekar PH, Van de Velde V, Mannens G, Edwards DJ. Itraconazole and hydroxyitraconazole serum concentrations are reduced more than tenfold by phenytoin. Clin Pharmacol Ther. 1995;58(6):617-24.
DOI: 10.1016/0009-9236(95)90017-9. PMID: 8529326.
3. Mouton JW, van Peer A, de Beule K, Van Vliet A, Donnelly JP, Soons PA. Pharmacokinetics of itraconazole and hydroxyitraconazole in healthy subjects after single and multiple doses of a novel formulation. Antimicrob Agents Chemother. 2006;50(12):4096-102.
DOI: 10.1128/AAC.00630-06.
4. Bae SK, Park SJ, Shim EJ, Mun JH, Kim EY, Shin JG, Shon JH. Increased oral bioavailability of itraconazole and its active metabolite, 7-hydroxyitraconazole, when coadministered with a vitamin C beverage in healthy participants. J Clin Pharmacol. 2011;51(3):444-51.
DOI: 10.1177/0091270010365557. Epub 2010 Apr 16. PMID: 20400647.
5. Schloer S, Brunotte L, Mecate-Zambrano A, Zheng S, Tang J, Ludwig S, Rescher U. Drug synergy of combinatory treatment with remdesivir and the repurposed drugs fluoxetine and itraconazole effectively impairs SARS-CoV-2 infection in vitro. Br J Pharmacol. 2021;178(11):2339-2350.
DOI: 10.1111/bph.15418.
6. Buchkowsky SS, Partovi N, Ensom MH. Clinical pharmacokinetic monitoring of itraconazole is warranted in only a subset of patients. Ther Drug Monit. 2005;27(3):322-33.
DOI: 10.1097/01.ftd.0000150135.2
7. Haria M, Bryson HM, Goa KL. Itraconazole. A reappraisal of its pharmacological properties and therapeutic use in the management of superficial fungal infections. Drugs. 1996;51(4):585-620. DOI: 10.2165/00003495-199651040-00006.
8. Bellmann R, Smuszkiwicz P. Pharmacokinetics of antifungal drugs: practical implications for optimized treatment of patients. Infection. 2017;45(6):737-779.
DOI: 10.1007/s15010-017-1042-z.

9. Mary Wharton, Michael Geary, Paul Sweetman, Laura Curtin, Niamh O'Connor, Rapid Liquid Chromatographic Determination of Itraconazole and its Production Impurities, *Journal of Chromatographic Science*. 2014;52(3):187-194
Available:<https://doi.org/10.1093/chromsci/bmt009>.
10. Hurtado FK, Souza MJ, Melo J de & Rolim Clarice M B, Microbiological assay and HPLC method for the determination of fluconazole in pharmaceutical injectable formulations. *Lat Am J Pharm*. 2008; 27:224.
11. Marchetti O, Majcherczyk Paul A, Glauser Michel P, Bille J, Moreillon P & Sanglard D, Sensitive bioassay for determination of fluconazole concentrations in plasma using a *Candida albicans* mutant hypersusceptible to azoles. *Antimicrob Agents Chemother*. 2001;45:696.
12. Amantea MA, Stevens DA & Bennett JE, Standardization of a fluconazole bioassay and correlation of results with those obtained by high-pressure liquid chromatography. *Antimicrob Agents Chemother*. 1991;35:846.
13. Mendes FES, Oliveira LVN, Fri ES, Alvarenga DG, Pinto MR, Taborda CP, Soares BM, Cisalpino PS & Santos DA, Correlation of the in vitro antifungal drug susceptibility with the in vivo activity of fluconazole in a murine model of cerebral infection caused by *Cryptococcus gattii*. *Eur J Clin Microbiol Infect Dis*. 2010;29:1525.
14. Rewak-Soroczynska J, Sobierajska P, Targonska S, Piecuch A, Grosman L, Rachuna J, Wasik S, Arabski M, Ogorek R, Wiglusz RJ. New Approach to Antifungal Activity of Fluconazole Incorporated into the Porous 6-Anhydro- α -L-Galacto- β -D-Galactan Structures Modified with Nanohydroxyapatite for Chronic-Wound Treatments—In Vitro Evaluation. *Int. J. Mol. Sci*. 2021;22:3112.

© 2021 Venugopal and Reddy; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<https://www.sdiarticle4.com/review-history/76665>