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SYNTHESIS AND EVALUATION OF NANOSTRUCTURED PARTICLES OF SALT OF KETOCONAZOLE FOR SOLUBILITY ENHANCEMENT

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The purpose of this work was to apply approach of salt formation for improvement of solubility and dissolution rate of Ketoconazole, a water insoluble antifungal drug. Ketoconazole dihydrochloride was synthesized by simple bubbling anhydrous hydrogen chloride gas into the acetonic suspension of ketoconazole. According to previous reports, the technique produced clustures of nanoparticles during crystallization, which contributes to solubility enhancement. The elemental analysis elaborated the molecular formula of $C_{26}H_{28}Cl_2N_4O_4 \cdot 2HCl$ and its structure was confirmed by gas chromatography-mass spectroscopy (GC-MS), Fourier transform infrared (FTIR), UV- spectroscopic characterization and differential scanning calorimetry (DSC). The morphological study by scanning electron microscopy (SEM) showed that the salt particles were as clusters of dispersible nanoparticles. Aqueous solubility measurements showed that aqueous solubility of the salt was extremely greater than its base. The percent dissolution of ketoconazole out of the salt after 15 min was also found more than 90%. The stability studies for 6 months were carried out for assay and dissolution which indicated that the salt remained stable. The present results depict that hydrochloride formation can significantly improve solubility and dissolution rate of ketoconazole and the method would be an easy, economical and practical alternative to the commercially available ketoconazole formulations.

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Keywords: Antifungal, Ketoconazole, Nanocrystals, Salt formation, Solubility enhancement

1. Introduction

Ketoconazole ($C_{26}H_{28}Cl_2N_4O_4$) is 1-Acetyl-4-[4-[[(2RS, 4SR)-2-(2, 4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl) 1, 3-dioxolan-4-yl] methoxy] phenyl] piperazine [1]. Ketoconazole (KTZ) is an active triazole antifungal agent used against variety of fungal strains. KTZ inhibits growth of dermatophytes and yeast species such as *Candida albicans* [2, 3]. A very hydrophobic characteristics and extremely weak basicity of KTZ contributes to its poor aqueous solubility [4].

Salt formation has been the commonest approach of enhancing aqueous solubility, dissolution rate of poorly water soluble drugs for application in solid dosage forms, parenteral as well as other liquid or semisolid formulations. Of about 300 new chemical entities approved by the FDA during the 12 years from 1995 to 2006 for marketing, 120 were in salt forms. Also, amongst the 101 approved salts of basic drugs, 54 salts were prepared with hydrochloric acid, demonstrating the hydrochloride was the major salt form [5].

The purpose of present work was to explore the feasibility of the hydrochloride salt to improve the solubility and dissolution rate of KTZ. Improvement of KTZ solubility can not only

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assist the oral use of it but also can enhance the applicability of KTZ in anti-dandruff shampoo preparations to make them cost effective as well as translucent and elegant.

Ketoconazole salt was prepared by bubbling anhydrous hydrogen chloride gas into suspension of KTZ in acetone, and characterized by Gas chromatography- Mass Spectroscopy (GC-MS), scanning electron microscopy (SEM), X-ray Diffractometry (XRD) and differential scanning calorimetry (DSC). The prepared salt form was evaluated for solubility, *in vitro* dissolution, *in vitro* antifungal activity and stability.

2. Materials and methods

2.1 Materials

Ketoconazole was supplied by Ajanta Pharma, Mumbai. All other chemicals were of analytical grade. Samples of isolated strains of *Candida albicans* and *Aspergillus niger* were kindly provided by Dept. of Microbiology, V.M. Medical College, Solapur, Maharashtra.

2.2 Preparation of ketoconazole dihydrochloride

Synthesis of salt form of ketoconazole was carried out by simple gas bubbling method. Ketoconazole (10 g) was suspended in 200 ml of acetone. Into the suspension heated under reflux, the anhydrous gas of hydrogen chloride was slowly bubbled. The suspension turned into a clear solution after about 30 min. A pale yellow to orange precipitate was observed in another 5 to 10 min. The passage of hydrogen chloride gas lasted for 30 min and the mixture was allowed to stand overnight at room temperature. The product was collected by filtration, washed with acetone and dried at 105°C. The proposed scheme for salt synthesis is depicted in Fig. 1.

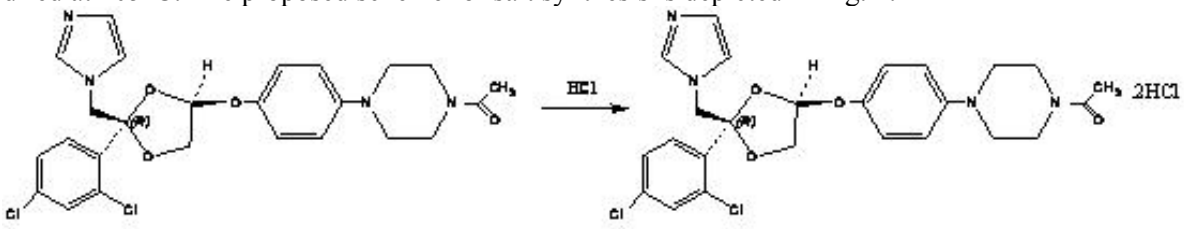

Fig. 1. Schematic representation for synthesis of Ketoconazole dihydrochloride

2.3 Determination of melting points

Primary assessment of changes after the chemical treatment was done by determination of melting points of KTZ and treated KTZ. Melting points were checked by conventional capillary method and are reported uncorrected.

2.4 Elemental analysis

Elemental Analyses of KTZ and treated KTZ were performed on Analytischer Funktionstest Vario EL Fab-Nr.11975059 for the calculation of contribution by C, H, N and O.

2.5 Fourier Transform Infrared (FTIR) spectroscopy

FTIR absorption spectra of KTZ and treated KTZ were recorded using a spectrometer (Nexus 670, Nicolet Instrument Co.) equipped with a DTGS detector. KBr disks were prepared (2 mg sample in 200 mg KCl) and scanned over a range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.6 Differential scanning calorimetry (DSC)

Thermal behavior of KTZ and treated KTZ were determined by means of Differential Scanning Calorimeter (DSC-7, PerkinElmer Inc.) with nitrogen flow rate of 40 ml/min and a heating rate of 10°C/min from 25 to 400°C.

2.7 Gas Chromatography- Mass spectroscopy (GC-MS)

GC/MS analysis of KTZ and salt was performed on a SGE Fort GC capillary column- BP-1 (30 m × 0.25 μm internal diameter, film thickness 0.25 μm) composed of 100% dimethyl

polysiloxane as stationary phase. For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as the carrier gas at a constant flow rate of 1 ml/min. Injector and MS transfer line temperature were set at 260°C and 320°C respectively. The oven temperature was programmed from 60 - 320°C at 3°C/min, increase then held isothermal for 11 min and finally raised to 320°C at 10°C/min. Diluted samples (1/100, v/v in methanol) of 1.0l were injected manually in the split less mode. Software adopted to handle mass spectra and chromatograms was a ChemStation.

2.8 Scanning electron microscopy (SEM)

SEM photomicrographs were taken to compare the crystal morphology of KTZ and KTZ salt. SEM micrographs were taken using scanning electron microscopy (XL30, Philips Analytical Inc.). Samples were coated with gold before examination.

2.9 UV spectroscopy

The drug and salt were analyzed for the UV spectra (Systronics Double Beam Spectrophotometer- 2201). Determination of absorption maxima was performed for KTZ and its salt in 0.1 N HCl. Quantitative analysis of KTZ in solubility and dissolution testing were performed by UV spectrophotometry at 227.2 nm with the spectrophotometric determination. Different spectrophotometric parameters of drug and salt like absorption maxima, absorption spectra, Sandel's sensitivity and molar extinction coefficients were compared. Molar absorptivities were estimated by preparing 0.01mM solutions in 0.1N HCl.

2.10 Solubility measurement

Solubility determination of KTZ and salt was done by Higuchi-Connor's method [6]. Excess amounts of KTZ and KTZ dihydrochloride were added to 10 ml of distilled water. The suspensions were shaken on a rotary shaker at constant temperature (25 ± 0.5 °C) for 48 hrs. The vials were allowed to stand for 24 hrs for equilibration. The samples were filtered through a Whatman filter paper and appropriately diluted with distilled water. The amount of dissolved KTZ was quantified by UV spectroscopy at 227.2 nm.

2.11 Dissolution testing

Dissolution profiles of KTZ and salt were determined in 900 ml of 0.1 N HCl (pH 1.2) at 37 ± 1°C using USP-XXIV Type-II paddle dissolution test apparatus (Electrolab TDT-06P, India) at 100 rpm. The accurately weighed samples equivalent 100 mg of KTZ were filled in size '0' gelatin capsules. 5 ml aliquots were periodically collected and filtered through 0.45µ membrane filters maintaining sink condition and analyzed at 227.2 nm after suitable dilution [7].

2.12 *In vitro* antifungal study

An agar diffusion method [3] was used for the determination of antifungal activity of KTZ and KTZ salt. Standard Petri dishes (9 cm diameter) containing medium to a depth of 0.5 cm were used. The sterility of the lots was controlled before use. Inocula were prepared by suspending 1-2 colonies from 24 h cultures in Sabouraud's medium into tubes containing 10 ml of sterile saline. The tubes were diluted with saline. The inoculum (0.5 ml) was spread over the surface of agar and the plates were dried at 35°C for 15 min prior to placing the antifungal samples. The bores of 0.5 cm diameter were prepared and 20 µl samples of test solutions (2% w/v) were added in the bores. After incubation at 35°C for 24 h, the halos of inhibition around the bores were measured. Experiments were performed in triplicates.

2.13 Stability testing

Stability testing was conducted under the storage condition of 25 ± 2 ºC/65 ± 5% RH. Capsules filled with KTZ dihydrochloride were packaged in closed aluminum-polyethylene laminated bags. Physical examination and assay were evaluated at fixed time intervals at 0, 1, 2, 3, and 6 months.

3. Results

3.1 Salt formation

A pale yellowish to orange colored powder was obtained with a good yield of about 96%. Elementary analysis (results presented as % of element found/theoretically calculated) based on $C_{26}H_{28}Cl_2N_4O_4 \cdot 2HCl$, shows C: 51.56/51.62; H: 5.327/4.963; N: 9.225/9.265. The results of elemental analysis suggest the evidence of dihydrochloride salt formation.

3.2 FTIR spectra

FTIR spectra (Fig. 2) of KTZ and KTZ dihydrochloride show that the characteristic absorption peak of hydrochloride salt was observed at 2366.7 cm^{-1} and 3416cm^{-1} might be attributed to O-H bond stretching vibration, implying a small amount of adsorbed water in the sample. The other characteristic absorptions in the FTIR spectra of KTZ dihydrochloride were similar to those of KTZ suggesting that all other structural features required for the antifungal activity are not affected by the reaction.

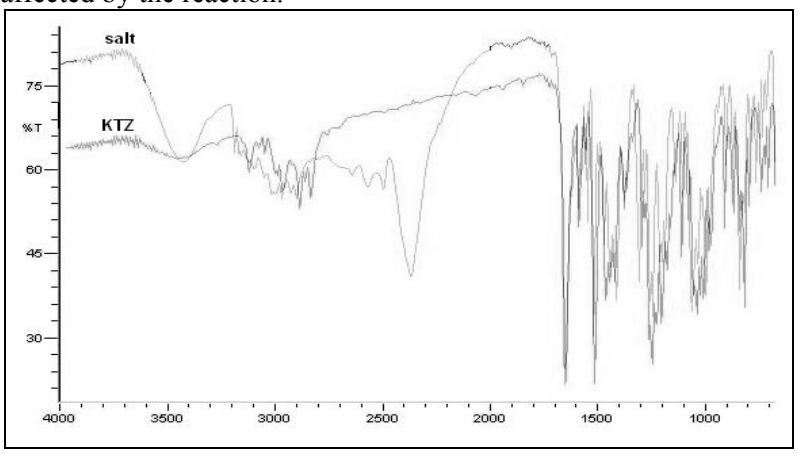

Fig. 2. IR spectra of KTZ and Salt

3.3 DSC curves

Fig. 3 shows the DSC curves of KTZ and KTZ dihydrochloride. KTZ was characterized by a single, sharp melting endotherm at 148.12°C (ΔH=-106.5J/g), in agreement with its melting point. The salt showed an endothermic peak at 229.67°C (ΔH= - 44.77 J/g). This result supports melting point determination of KTZ salt.

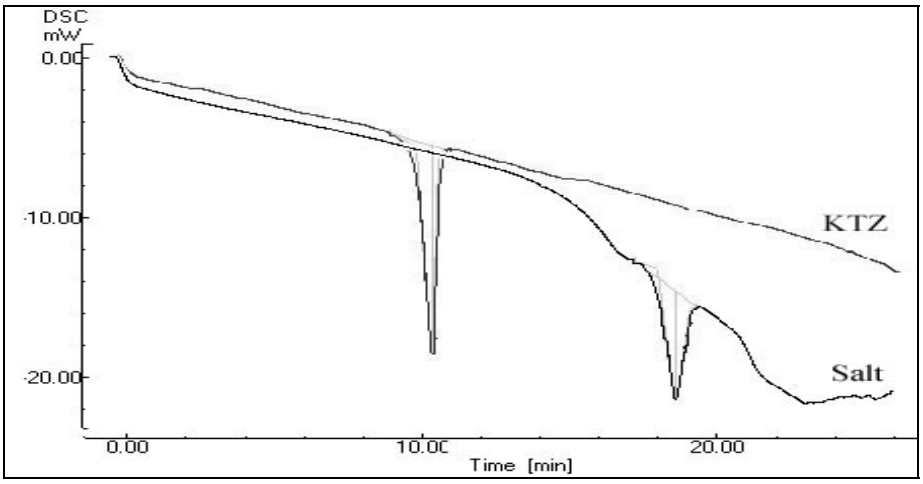

Fig. 3. DSC study of KTZ and salt

3.4 Gas chromatography- Mass spectrum

Gas chromatography was performed using non-polar stationary phase. Chromatograms of drug and salt demonstrate the difference in the elution time, where, salt having shorter elution time than KTZ indicating increased polarity of KTZ in its salt form. The mass spectrum (Fig. 4) of salt shows the presence of molecular peak at m/e = 606.8, suggesting the increment in the molecular weight of the KTZ by dihydrochloride salt formation.

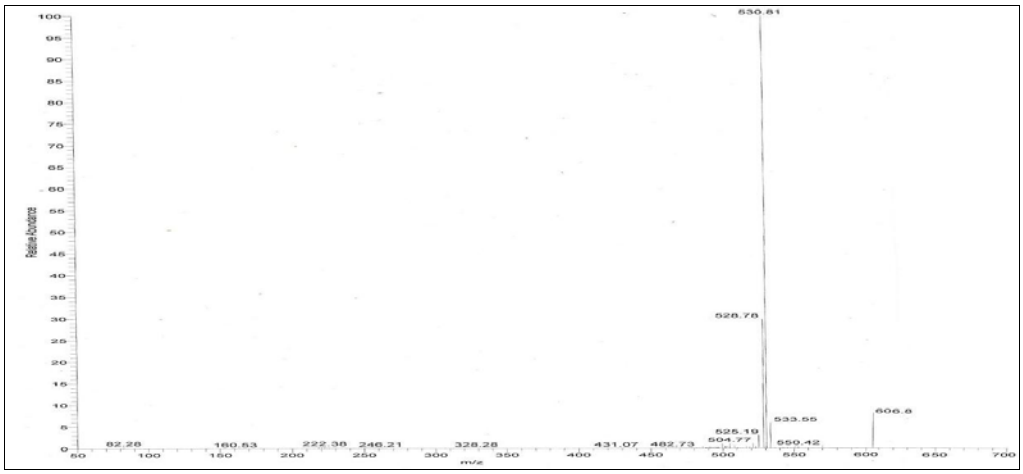

Fig. 4. Mass Spectrum of Salt

3.5 UV Spectrophotometric Study

The UV- spectrophotometric absorption is a characteristic of the basic structure and absorbing groups attached to the basic skeleton. Depending on this fact, spectrophotometric properties like UV spectra, Molar absorptivity of both the compounds were compared. It was found that UV spectra of KTZ and salt exactly overlap to each other suggesting the intactness of basic chromophore of KTZ structure in its salt form (Fig 5). Moreover, the absorbance intensity in salt was found less in salt than parent form indicating the less molar content of the absorbing species i.e. KTZ. Molar extinction coefficients were found to be similar. This result suggests that 607.2 moles of KTZ salt are equivalent to 532.2 moles of KTZ, supporting the 'dihydrochloride' salt formation. Different spectrophotometric parameters are shown in Table 1.

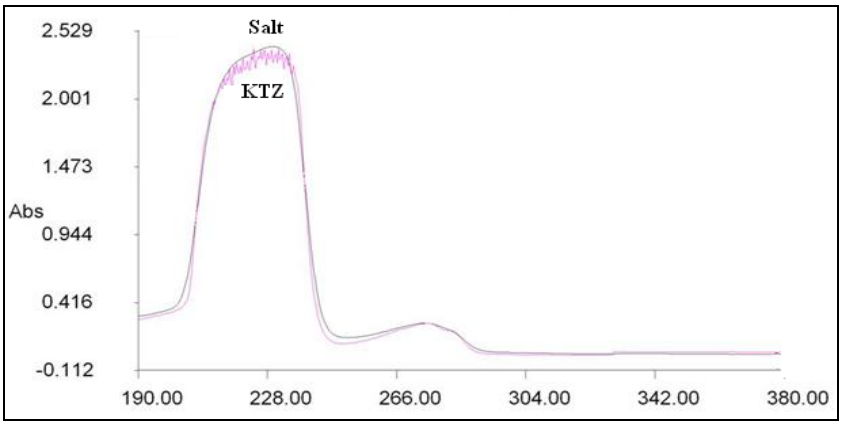

Fig. 5. UV Spectra of KTZ and Salt

3.6 SEM study

The photomicrograph showed the morphological and micromeritic characteristics of KTZ and its salt form (Fig. 6). Particles of KTZ were observed as solid rod shape of size ranging from 6 to 36 µm (Fig. 6a and 6b). In contrast, the morphology of salt was found as the clustures of aggregated nanoparticles (Fig. 6c). The high magnification SEM morphology (Fig 6d) revealed

salt particles are present with a geometric diameter of <1000 nm. The nano-structure of salt particles can be a contributing factor for solubility enhancement of poorly soluble KTZ due to increased effective surface area as well as surface free energy. Nano sizing also effects on the dispersibility and wettability of the particles.

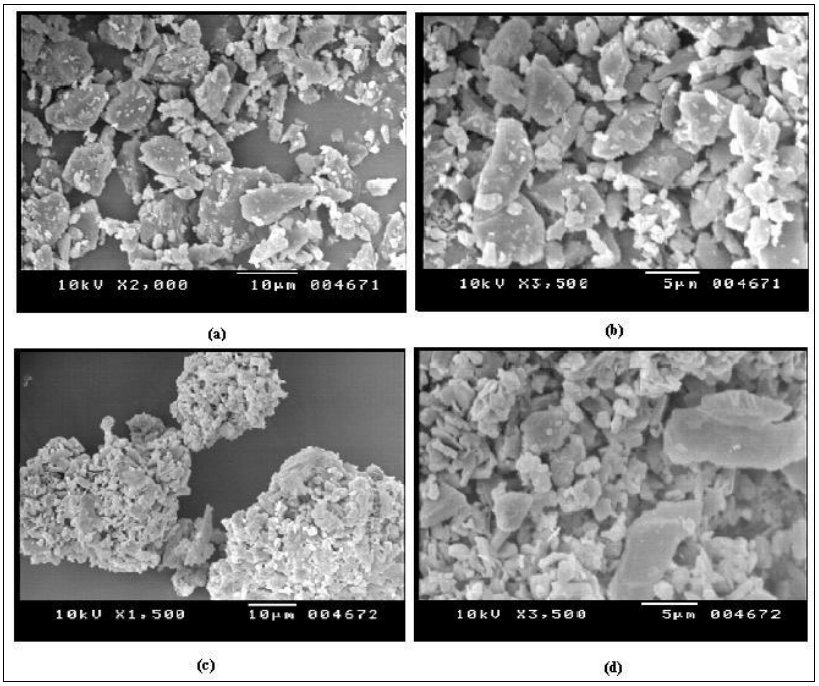

Fig. 6. SEM microphotographs of KTZ and salt

3.7 Solubility study

Hydrochloride salt formation significantly improves the solubility of KTZ in distilled water as well as 0.1N hydrochloric acid at 25°C. The solubility of KTZ was found to be 24.29 ± 1.47 µg/ml, which is in good agreement with the reported value [8]. The aqueous solubility of dihydrochloride salt was about 1.417 ± 0.34 g/ml which shows a tremendous enhancement of intrinsic solubility of salt compared to its base. The enhanced solubility can be correlated with salt formation as well as nano-particulate structure of prepared compound giving rise to increased effective surface area as well as increased dispersibility. Thus salt particles can get easily dispersed and immediately dissolved in presence of solvent.

3.8 Dissolution testing

Fig. 7 demonstrates the dissolution profiles KTZ and its salt. Less than 20% of KTZ was dissolved after 120 min. While dissolution profile of hydrochloride salt form of KTZ that more than 90% of KTZ was dissolved from KTZ dihydrochloride within first 15 min and a complete dissolution was observed in 30 min.

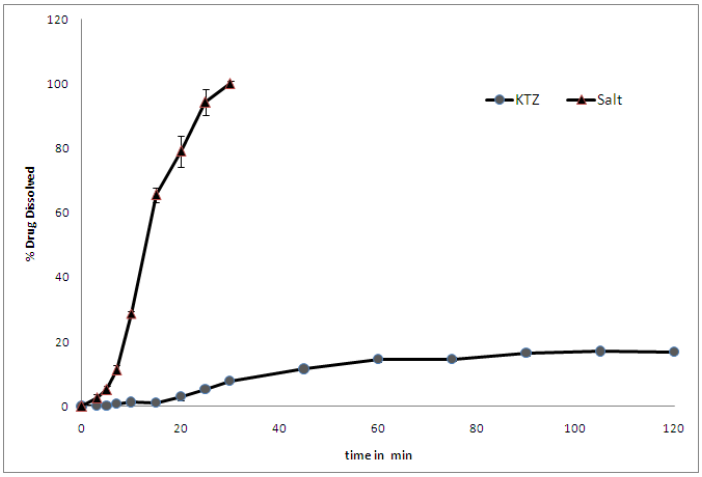

Fig. 7. Dissolution profiles of KTZ and Salt

Table 1. Comparison of evaluation parameters for KTZ and salt

Evaluation Parameter	KTZ	Salt
Aqueous Solubility	24.288 ± 1.47µg/ml	1.417 ± 0.34 g/ml
$\%R_{15\ min}$	1.203 ± 0.36	65.537 ± 2.34
$\%R_{30\ min}$	7.783 ± 0.24	99.925 ± 0.91
Molar Absorptivity (lit/mole/cm)	30145.57	29356.56
Sandals Sensitivity (mcg/Sq.cm/0.001)	0.017628	0.020588

3.9 *In vitro* antifungal activity:

In vitro antifungal activity on three different fungal species viz. *Candida albicans*, *Aspargillus niger*, *A. flavan* was done by agar diffusion method. As depicted by Fig. 8, drug and salt showed similar zones of fungal growth inhibitions thus the *in vitro* antifungal efficacy of the KTZ was not significantly affected by the salt formation, suggesting the intactness of the necessary structural characteristics for antifungal activity.

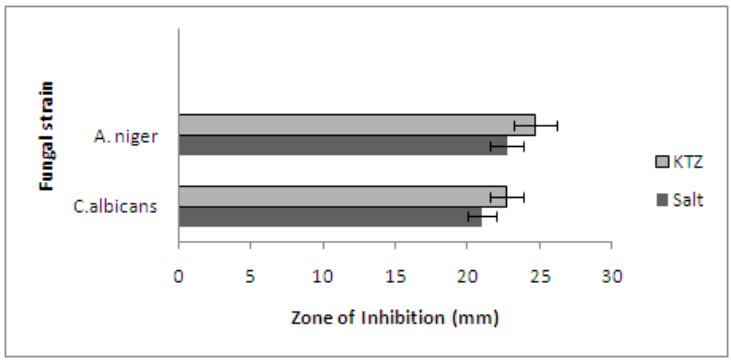

Fig. 8. Antifungal activity of KTZ and Salt

3.10 Stability study

Accelerated storage condition for 6 months caused no significant change in assay, related substances, solubility and dissolution. This might be due to increase in the melting point of substance which contributes to enhanced stability. The results suggested that prepared KTZ dihydrochloride is chemically and physically stable.

4. Conclusion

Based on the present results, it can be concluded that formation of hydrochloride salt can significantly improve the solubility and dissolution rate of KTZ without disturbing its basic essential structure and antifungal activity. The approach can be said as simple and industrially applicable.

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