Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

PREPARATION AND ANTIBACTERIAL CHARACTERISTICS OF MIXED CRYSTALS OF TUTTON'S SALT $(NH_4)KZn(SO_4)_2{\cdot}6H_2O$

Binitha S. D.*[1], V.T. Jisha[2]

[1]Department of Chemistry, Christian College Kattakada, Kerala, India
[2]Department of Physics, Christian College Kattakada, Kerala, India
*Corresponding author: sdbinitha1993@gmail.com

ABSTRACT

Mixed crystals of the Tutton's salt family with general chemical formula $(NH_4)K_xZn_{(1-x)}(SO_4)_2{\cdot}6H_2O$ were grown by employing slow evaporation solution growth technique. The major aim of the study was to develop an efficient antibacterial for preventing the spread of new diseases. The coexistence of potassium and zinc ions in the mixed crystals were characterized by different instrumental techniques such as X-ray Diffraction (XRD) and Fourier Transform Infrared (FT-IR) spectroscopy. The crystal structure had been affirmed by XRD and the pattern was discovered to be in the middle of ammonium zinc sulfate hexahydrate (AZSH) and potassium zinc sulfate hexahydrate crystals (KZSH). The study confirmed that ammonium potassium zinc sulfate hexahydrate (AKZSH) crystal belongs to monoclinic space group $P2_1/a$. FT-IR spectral analysis was done to study the vibrational bands of AKZSH crystal and assigned the modes of vibration to different functional groups. In addition, antibacterial activity of the crystal was analyzed using agar well-diffusion method. The antibacterial activity of methanol and aqueous extracts of AKZSH were investigated against selected human pathogens such as *Escherichia coli* (gram-negative) and *Streptococcus* sp (gram-positive). It is evident from the results that, the gram-positive strain was found to be more sensitive than the gram-negative strain with excellent and long-term antibacterial activity.

Keywords: Ammonium potassium zinc sulfate hexahydrate, Characterisation, XRD, FT-IR, Antibacterial activity.

1. INTRODUCTION

Recently our scientific interest and efforts have been concentrated in the study on growth and characterization of Tutton's salt due to its potential technological applications such as chemical energy storage, ultraviolet (UV) light filters, UV made sensors, spaceships, solar energy absorber and even in missile approach warning systems [1, 2]. The crystals of Tutton's salt are used to locate and track the sources of ultraviolet energy [3] and sometimes they are used to block UV radiation. Tutton's salt doped with rare earth ions are of particular interest for optical applications, such as solid-state lasers [4-7]. Most recently, Tutton's salts acts as promising materials for applications as ion-conductors in batteries, electrodes, cathodes, solid electrolytes and even in lithium or sodium batteries [8]. Tutton's salt are the isomorphic hydrated complexes with empirical formula $M_2M'(SO_4)_2{\cdot}6H_2O$ or $M_2M'(SeO_4)_2{\cdot}6H_2O$, (M = Cs, K, NH_4, Rb, Tl; M' = Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, V, Zn) [9]. The family of Tutton's salt crystallize in the monoclinic system with centrosymmetric space group $P2_1/c$ [Z = 2] [10]. This crystal contains two octahedral hexahydrate complexes $[M'(H_2O)_6]^{2+}$ in the crystal unit cell, Where M' is a bivalent cation and M is a monovalent cation. The octahedral groups are distorted by the Jahn-Teller effect.

The chips away at Tutton salt gems are rich and more crystals are accounted for at a consistent rate. Potassium and ammonium zinc sulfate hydrate crystals are the members of the Tutton crystalline salts, and they crystallize in the monoclinic crystal system. These crystals may merit important consideration because of their growth with high purity and suitable crystal sizes and small transparency in the UV region, and hence could be the potential candidates for some optoelectronic applications [11]. There are some recent studies on the growth, structural analysis, characterization and optical transmittance spectra of crystals. $K_2Ni_xCo_{1-x}(SO_4)_2{\cdot}6H_2O$ mixed crystals with good optical quality were obtained using the slow evaporation growth method by Pacheco et al [12]. Several other studies have also been reported in the synthesis and characterization of tutton's salt such as

Potassium manganese nickel sulphate hexahydrate [13], zinc magnesium ammonium sulfate hexahydrate [14], zinc nickel sulphate hexahydrate [15], zinc (II) doped ammonium nickel sulphate hexahydrate [16], potassium zinc nickel sulfate hexahydrate [17].

On the other hand, metal complexes that include sulfate have wide applications for the synthesis of drugs [18] due to their fungicidal-bactericidal and anticancer properties. In the past decades, plenty of people have suffered from diseases caused by unsafe drinking water and food containing bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. The traditional low molecular weight antibacterial materials have many disadvantages, such as toxicity to the environment and short-term antibacterial activity. Hence, there is an urgent need for the development of effective antibacterial materials [19]. In general, metal-based complexes have significant anti-bacterial, anticancer and antiviral effects [20]. Infectious diseases are the leading explanation for death world-wide. Antibiotic resistance has become a global concern. The clinical efficacy of many existing antibiotics is being threatened by emergence of multi drug resistant pathogens. There is continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action for new and re-emerging infectious diseases [21].

The aim of this paper is to carry out a crystal-chemical and antibacterial study of the synthetic compounds of general chemical formula $(NH_4)K_xZn_{(1-x)(}SO_4)_2\cdot 6H_2O$. In this work, the hexahydrate crystals of potassium and zinc-ammonium sulfate were synthesized by slow evaporation solution growth technique and its crystal structural parameters have been studied by XRD. The functional groups in the crystal have been analyzed by FT-IR spectroscopy. Further, antibacterial study was carried out by agar-well diffusion method with two human pathogens *Escherichia coli* (gram-negative) and *Streptococcus* sp (gram-positive) in comparison with a commercially available antibiotic, Amoxycillin.

2. MATERIAL AND METHODS

2.1. Synthesis

Crystals of AKZSH were grown by slow evaporation solution growth technique. For the synthesis of single crystal of AKZSH the precursors used are $ZnSO_4\cdot 7H_2O$, $(NH_4)_2SO_4$ and K_2SO_4 in a stoichiometric molar ratio given by the compositional form $(NH_4)K_xZn_{(1-x)(}SO_4)_2\cdot 6H_2O$ according to the following general equation:

$$ZnSO_4 + (NH_4)_2SO_4 + K_2SO_4 \longrightarrow (NH_4)K_xZn_{(1-x)(}SO_4)_2\cdot 6H_2O \text{ (AKZSH)}$$

The starting materials were dissolved in double distilled water and the solution was stirred for 2h for its homogeneity. Then it is heated to approximately 65˚C until the full dissolvent and a clear solution was obtained. This is the first step, which is the formation of the supersaturated solution, the solution was left to cool to room temperature and fast precipitation of small crystallites took place. The precipitated small clear crystallites were recrystallized many times following a similar procedure in a special crystallizing apparatus in which the solution temperature was fixed at 45°C. The resulting crystals, in equilibrium with the mother solution, were removed and dried. Large dimensions of single crystal was obtained after 5-7 weeks of crystallization and the high quality transparent crystals were harvested from the aqueous growth medium as shown in fig. 1 .

Fig. 1: Image of synthesized AKZSH crystal

2.2. Characterization

X-ray diffraction measurements were performed to analyze the crystalline structure of monocrystals using the Rigaku MiniFlex 600 X-ray diffractometer. The radiation used was CuKα with wavelength, λ = 1.5406 Å and energy, E = 8.04 keV at room temperature. The measurements were swapped from $2\theta = 10°$ to 80° with the step of 0.06°, copper target at 40kV, 30mA, and a scanning speed of 0.06°/min and incident wavelength $\lambda k\alpha$=1.5406 Å. A Perkin Elmer Fourier transform infrared spectrometer was used to record the FTIR spectra in the range 500-4500cm^{-1} by the KBr pellet method to study the functional groups of AKZSH crystal.

The antibacterial screening was determined using agar-well diffusion method. The methanolic and aqueous extracts of AKZSH were prepared by dissolving 100mg fine powder separately in 1 ml of methanol and water respectively. The contents were kept in shaker for 24 h (stock concentration of the extract: 100mg/mL). After

24 hours, the supernatant was filtered and used for the study. From the stock concentration (100 mg/mL), a lower concentration of 50 mg/mL was prepared. The inoculum of *Escherichia coli* and *Streptococcus* sp., were prepared in nutrient broth medium and kept incubation at 37˚C for 8 hours. After growth was observed, the cultures were stored in the refrigerator at 2-8˚C for analysis. Twenty ml of sterilized Muller Hinton Agar was poured into sterile petriplate, after solidification, 100 μl (10^5c.f.u./mL) of test organism were swabbed on the respective plates. Wells of 6 mm diameter were punched into the agar medium and filled with 100μL of plant extract (of 100 mg/mL and 50 mg/mL concentration), antibiotic solution (positive control) and solvent blank (methanol and water) (negative control).The plates were incubated for 24 hours at 37°C. After incubation the diameter of inhibitory zones formed around each discs were measured in cm and recorded.

3. RESULTS AND DISCUSSION

3.1. XRD

XRD was most widely used for the identification of crystal structure. Good monocrystal with natural and well-developed faces were obtained throughout the composition range and the structure of grown crystal has been analyzed by powder diffraction technique. XRD pattern of AKZSH crystal was shown in fig. 2. XRD measurements reveals that the crystal belongs to monoclinic system with centrosymmetric space group $P2_1/c$ and the cell parameters are a = 5.340 Å, b =9.524 Å, c =10.235 Å, α = γ =90º, β =100.070º, V =520.533 $Å^3$and Z =2. The strongest peaks observed were found at 2θ =21.54°, 29.29°, 40.91°, 43.20°, 52.89°, 57.80°, 63.63° and 74.58° related to the planes having miller indices (201), (200), (211), (301), (321), (411), (332), (413) and (502) respectively corresponding to lattice parameters a =5.340Å, b =9.524Å, c =10.235Å and α =90°, β =100.070°, γ =90°.

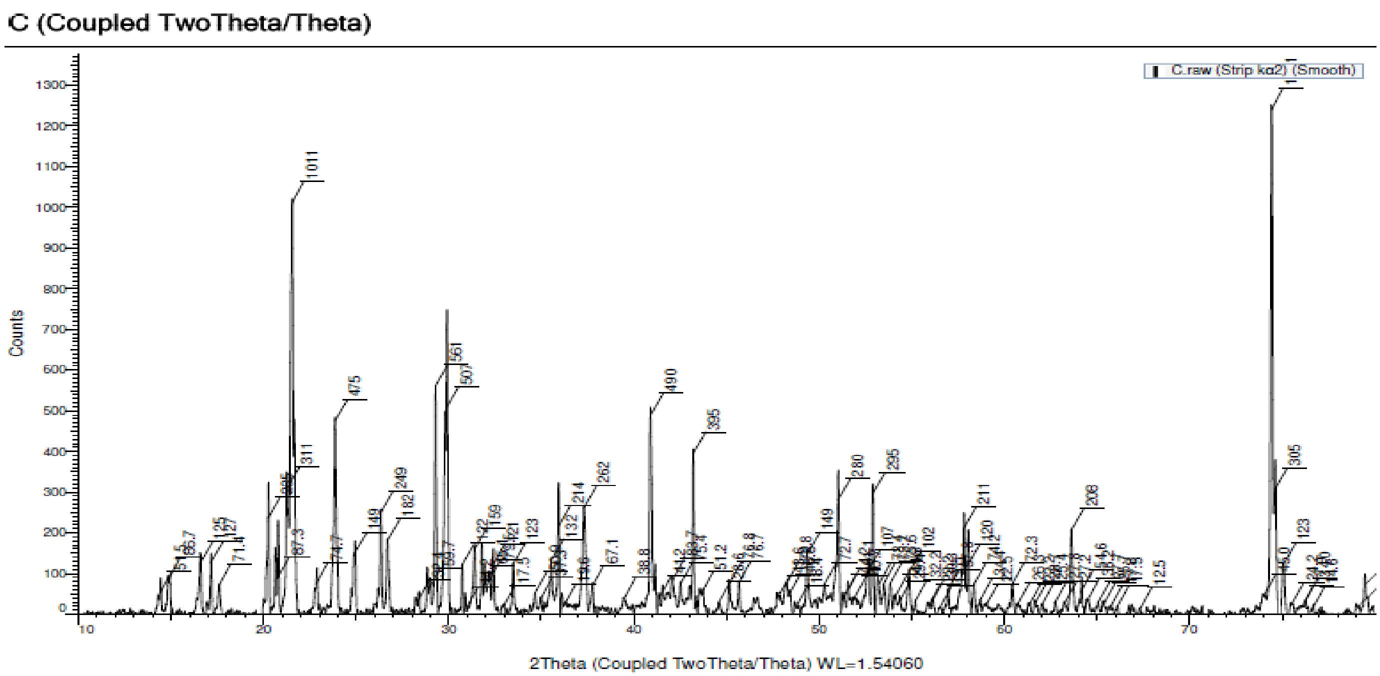

Fig. 2: XRD pattern AKZSH crystal

The cell parameters were fine conformity with the reported values with chemical formula $(NH_4)KZn(SO_4)_2·6H_2O$ [22]. Narrow peaks in powder diffraction study reveal the good crystallinity of the material. The refinement of the single crystal depicts that the asymmetric unit consists of Zn atom placed on the inversion center, one sulfate anion, three water molecules and the potassium and ammonium ions occupying the same sites. The Zn atom coordinates six water molecules with octahedral coordination while eight oxygen atoms coming from the sulfate anions and water molecules surround the potassium and nitrogen site. The SO_4^{2-} ions have tetrahedral geometry through O-H….O bonds and NH_4^+ are bonded with O of SO_4^{2-} with N-H…O hydrogen bonds. The oxygen atoms of the sulfate are receptors of six hydrogen bonds of the type O-H ··· O, where the water molecules participate as donors of H and also the oxygen atoms belonging to the sulfate are also receptors of eight hydrogen bonds of the type N-H ··· O where the molecules of NH_4 act as donors of H. The whole structure is repeated through alternative octahedral and tetrahedral layers. The crystalline size was calculated by using Debye Scherrer equation,

$$\tau = \frac{K\lambda}{\beta\cos\theta}$$

The crystalline size of prepared crystal was found to be 14 nm corresponding to the (201) plane. Our crystal parameters have been compared with monomeric crystals of KZSH and AZSH and understood that AKZSH is isomorphic to monomeric crystals [11]. Thus, the crystallographic data had a good approximation with the experimental diffraction peaks.

3.2. FT-IR spectroscopy

Fourier transform infrared spectroscopy was used for the identification of functional groups in the synthesized crystals. The spectrum was recorded in the spectral range of 500-4500 cm^{-1} shown in fig. 3. In this spectra, a very broad characteristic band of the water molecule was observed at v_1 = 3157.47 cm^{-1} that corresponds to the stretching vibrations of free OH groups [9]. A strong vibration band around v_2 = 1622.13 cm^{-1} is due to the in-plane bending vibration of water molecules caused by the rocking and scissoring of hydrogen atoms. The observation of bands in the spectral region of 1700-1400 cm^{-1} shows water being strongly hydrogen bonded in the crystal structure with different molecular bonds. The NH_4^+ tetrahedra present a very strong band at v_4 = 1400.32 cm^{-1} corresponding to the N-H bending mode. The SO_4^{2-} tetrahedra presents a strong peak at v_3 = 1134.14 cm^{-1} corresponding to the triply degenerated asymmetric stretching mode and a very strong peak at v_4 = 621.08 cm^{-1} corresponding to the triply degenerated bending mode [23]. The FTIR spectrum character of AKZSH lies in between the behavior of KZSH and AZSH and the active region of the sample is apparently from 1800-400 cm^{-1} [11]. The FTIR spectrum of AKZSH confirms that the grown crystal forms a combination of both potassium and ammonium based vibration bands.

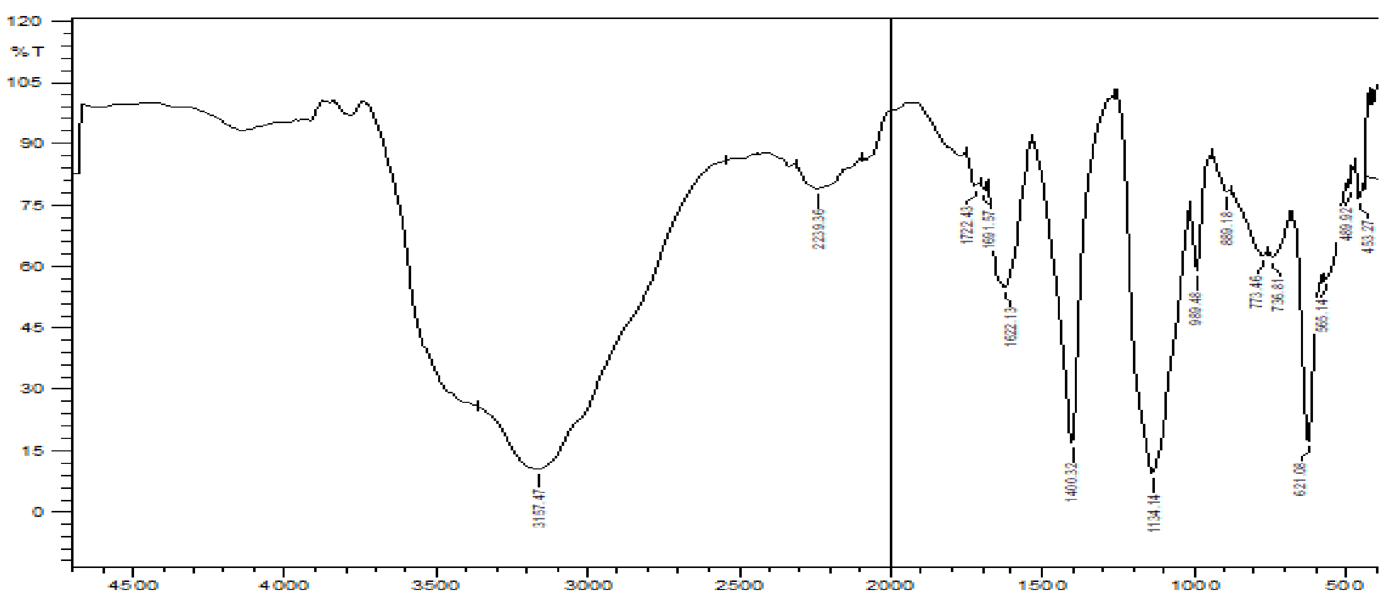

Fig. 3: IR spectrum AKZSH crystal

3.3. Antibacterial activity

The antimicrobial activity of methanol and aqueous extracts of AKZSH were investigated using agar-well diffusion method against selected human pathogens such as *Escherichia coli* and *Streptococcus* sp and the results were shown in tables 1 and 2. These two different pathogens have also tested with commercially available antibiotic (Amoxycillin) and results were indicated in table 3 and fig.4. Both the extracts of AKZSH used against the pathogenic organisms have showed varied degree of antimicrobial activity against the pathogens.

Antibacterial susceptibility testing of methanolic extract of AKZSH: The two concentrations of the extract were found to be effective against the selected strains

(*Streptococcus* and *E. coli*) because the growth of these organisms were inhibited by the extracts forming an inhibitory zone of different diameters. The highest inhibition was observed with *Streptococcus* which was found to be 2.4 cm at 100mg/ml concentration. Similarly, a good inhibitory zone (2.2 cm) was also observed with *E. coli* at 100mg/ml concentration. These results revealed the significant antibacterial activity of the extract against studied bacteria.

Antibacterial susceptibility testing of aqueous extract of AKZSH: The highest inhibition was observed with *Streptococcus* which was found to be 2 cm at 100mg/ml concentration. Similarly, a satisfactory inhibitory zone (1.9 cm) was also observed with *E. coli* at 100mg/ml concentration. These results revealed the significant antibacterial activity of the extract against studied bacteria. In this present study, as expected, the highest concentration of the extract (100mg/ml) showed the highest activity than the rest [20]. The preliminary screening for antimicrobial activity showed, that the methanolic extract of AKZSH exhibited maximum inhibitory zone (2.4 cm) against *Streptococcus* sp. and for *E.coli* a satisfactory zone of 2.2cm. While the aqueous extracts of AKZSH showed least inhibitory activity when compared with methanolic activity. Also, it is clear from the results that, the gram positive strain (*Streptococcus*) is found to be more sensitive than the gram negative strain (*E.coli*) because its zone of inhibition is higher than that of *E. coli*. Therefore, AKZSH has potential applications as a broad-spectrum antibacterial agent.

Table 1: Antibacterial activity of different concentrations (100mg/ml and 50mg/ml) of methanolic extracts of AKZSH by agar well-diffusion method

Sl. No	Organism	Concentration of the extract	Resistant	Sensitive	Zone diameter (in cm)
1.	*Escherichia coli*	100mg/ml		+	2.2
		50mg/ml		+	1.6
2.	*Streptococcus* sp.	100mg/ml		+	2.4
		50mg/ml		+	1.7

Table 2: Antibacterial activity of different concentrations (100mg/ml and 50mg/ml) of aqueous extracts of AKZSH by agar well-diffusion method

Sl.No	Organism	Concentration of the extract	Resistant	Sensitive	Zone diameter (in cm)
1.	*Escherichia coli*	100mg/ml		+	1.9
		50mg/ml		+	1.4
2.	*Streptococcus* sp.	100mg/ml		+	2.0
		50mg/ml		+	1.5

Table 3: Antimicrobial activity of standard antibiotic (Amoxycillin)

Organism	Zone diameter of amoxycillin
E. coli	3.5
Streptococcus sp.	3.1

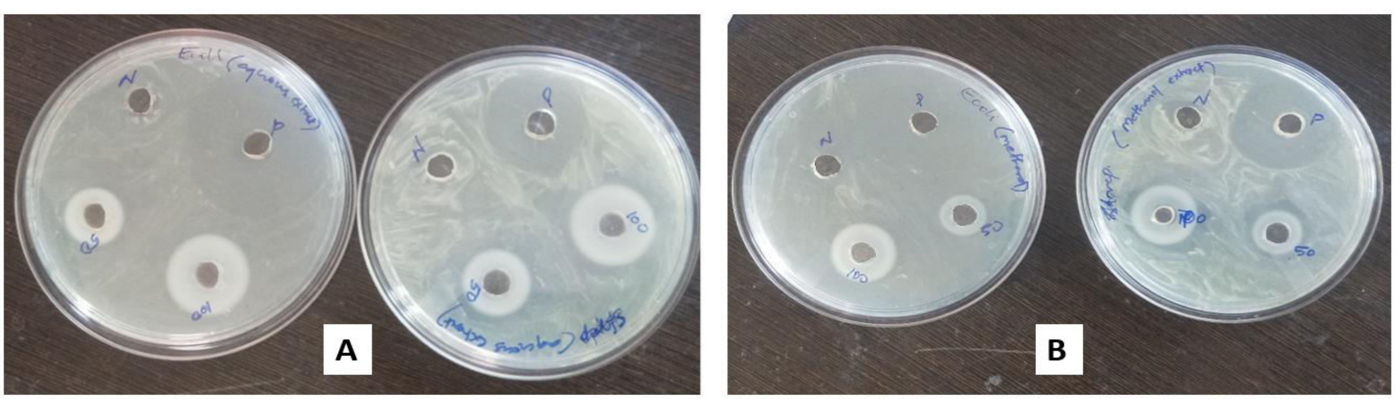

Fig. 4: Antibacterial activity of methanolic (A) and aqueous extracts (B) of AKZSH and standard antibiotic amoxicillin using agar well-diffusion method

4. CONCLUSION

The AKZSH crystal has been synthesized by slow evaporation growth technique and pure crystals with good crystallinity were obtained. The resultant crystal showed characteristic morphology of the Tutton's salts with empirical formula $(NH_4)K_xZn_{(1-x)(}SO_4)_2 \cdot 6H_2O$ and has been put under successive investigations to study their structural properties. XRD study was carried out and showed that the crystal has a pattern, which may be considered as a combination of KZSH and AZSH single crystals. The refinement of the diffraction pattern revealed that the crystal has monoclinic crystal system with space group $P2_1/c$ and the lattice constants were median between the values of the lattice constant of KZSH and AZSH single crystals. FTIR spectroscopic analysis revealed that AKZSH crystals had the spectral features of KZSH and AZSH crystals with small deviations. FTIR spectrum of AKZSH identified the functional groups present in it. The vibrational modes of octahedral complexes, NH_4^+ and SO_4^{2-} tetrahedra are obtained from FTIR. AKZSH crystal was assayed for its antibacterial activity and results obtained gives a scope for future pharmacological applications in designing better and more active drugs.

5. ACKNOWLEDGEMENTS

We acknowledge Department of Chemistry Christian College Kattakada for providing facilities to do the research work.

Conflict of interests

The authors declare that they have no conflict of interests.

6. REFERENCES

1. Qian X, Liu H. *Adv Mater Res*, 2011; **332-334:** 1687-1690.
2. Manomenova VL, Rudneva EB, Voloshin AE. *Russian Chem Rev,* 2016; **85:**585.
3. Singh NB, Partlow WD, Strauch S, Stewart AM, Jacovitz JF, Coffey DW et al. Google Patents; 1998.
4. Soumati A, Martin IR, Zayani L, Hernandez-Rodriguez MA, Soler-Carracedo K, Lozano-Gorrin AD et al. *J Lumin,* 2016; **177:**93-98.
5. Soumati A, Zayani L, Palomino JM, Cruz-Yusta M, Vicente CP. *Therm Anal Calorim,* 2015; **122:** 929-936.
6. Soumati A, Martin IR, Zayani L, d.Lozano-Gorrin AD, Ben Hassen Chehimi. *J Lumin,* 2017; **188:**148-153.
7. Soumati A, Martin IR, Zayani L, Hernandez-Rodriguez MA,. Soler-Carracedo K, Lozano-Gorrin AD et al. *Opt Mater,* 2016; **53:**190-194.
8. Bejaoui A, Souamti A, Kahlaoui M, Lozano-Gorrin AD, Morales Palomino J, Ben Hassen Chehimi D. *Mater Sci Eng B,* 2019; **240:**97-105.
9. Santunu Ghosh, Michelle Oliveira, Tiago S Pacheco, Genivaldo J Perpetuo, Carlos J Franco. *J Cryst Growth,* 2018; **487:**104-115.
10. Montgomery H, Lingafelter EC. *Acta Crystallogr,* 1964; **17:**1478-1479.
11. Abu El-Fadl A, Nashaat AM. *Appl Phys A,* 2017; **123:**1-11.
12. Pacheco TS, Ghosh S, De Oliveira M, Barbosa AA. *J Sci Adv Mater Dev,* 2017; **2:**354-359.
13. Masilamani V, Shanthi J, Sheelarani V. *Crystallogr Rep,* 2014; **59:**1114-1117.
14. Ramaswamy G, Bhagavannarayana G, Madhurambal G, Meenakshisundaram S. *J Cryst Growth,* 2012; **352:**137-142.
15. Zhuang X, Su G, Wang G, Li G, Huang Z. *Cryst Res Technol,* 2004; **39:**754-758.
16. Baviskar SJ, Kale MS, Bhasvar DS. *Integer J Engg Res Tech,* 2014; **15:**171-175.
17. Vanitha D, Kumar SS, Athimoolam S, Bahadur SA. *Optik-Internat J Light electron Opt,* 2015; **126:**4553-4556.
18. Jain M, Singh RV. *Bioinorg Chem Appl,* 2004; **2006:** 1-10.
19. Qingguo Meng, Lintong Wang, Dongfang Wang, Jianjian Yang, Chen Yue, Jitao Lu. *Crystals,* 2017; **7:**112-119.
20. Sagunthala P, Veeravazhuthi V, Yasotha P, Hemalatha P. *World J Pharm Res,* 2016; **5 Suppl 8:**414-425.
21. Baviskar SJ, Bhavsar DS. *Arch Appl Sci Res,* 2011; **3 Suppl 3:**279-2866.
22. JCPDS data card no 34-025.
23. Nakamoto K. Theory of Normal Vibrations, Infrared and Raman Spectra of Inorganic and Coordination compounds, John Wiley & Sons; 2008. p. 1-147.