

Growth and Characterization of Oxalic Acid Doped with Tryptophan Crystal for Antimicrobial Activity

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ABSTRACT

Single crystals of pure oxalic acid and oxalic acid doped with tryptophan have been grown by slow evaporation method. These crystals are characterized by single crystal XRD, Powder XRD, FT-IR and UV-Visible spectral analyses. Effect of tryptophan as an impurity on the properties of oxalic acid has also been investigated. X-ray diffraction analysis indicates the crystal system as monoclinic. The functional groups have been identified using Fourier transform infrared spectral analysis. The UV-Vis spectral analysis shows that these crystals have wide transparency range in the entire visible region. The pure and doped crystals were analysed by the antimicrobial activity study against the gram positive and gram negative bacteria by disk diffusion method.

Keywords: Antimicrobial activity, Oxalic acid, Tryptophan, XRD, FT-IR, UV-Visible spectroscopy

INTRODUCTION

The organic compound of oxalic acid is act as a hydrogen-bonded material in which two carboxyl groups are joined directly [1,2]. Oxalic acid, also known as ethanedioic acid, is a naturally occurring compound which is found in many different types of vegetables. For humans, oral and topical applications of this acid are highly toxic to the body due to its bleach-like and corrosive properties [3]. The addition of amino acids in the organic material may perform changes in the lattices of crystal behavior. The effect of amino acid as dopant on the growth and the properties of single crystal were investigated by many researchers [4-9]. In the similar manner the effect of tryptophan as additives on the growth of oxalic acid was investigated in present study by employing various characterization techniques such as single crystal XRD, powder XRD, FT-IR, UV-Visible spectroscopy and antimicrobial activity studies.

MATERIALS AND METHODS

Materials

The materials used for this crystallization are oxalic acid; deionized water and tryptophan were purchased from the Merck, India Ltd, Mumbai.

Slow evaporation method

Analytical Reagent grade of oxalic acid and tryptophan are taken in the equimolar ratio (1:1) and dissolved in doubly deionized water. The solution was stirred well at constant rate to get homogeneity. The solution was transferred to a beaker and it was allowed to evaporate at room temperature for a few days to get the crystal. Similar procedure was followed to obtain the pure oxalic acid. Good quality crystals of pure and doped crystals are yielded within a period of 15 to 20 days. The photographic view of the grown crystals is shown in **Figure 1**.

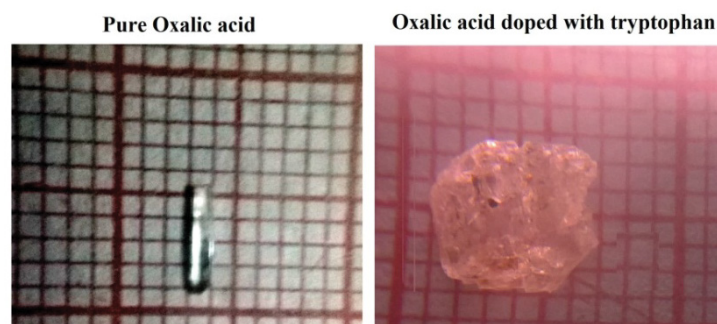


Figure 1: Photographic view of pure oxalic acid and oxalic acid doped with tryptophan crystals

RESULTS AND DISCUSSION

Single crystal XRD analyzes

The measurement of unit cell dimensions of pure oxalic acid and oxalic acid doped with tryptophan crystals are carried out using the SMARTAPEX CCD area-detector diffractometer with Mo K α radiation ($\lambda=0.71073 \text{ \AA}$). It gives the unit cell parameter values of the pure oxalic acid and oxalic acid doped with tryptophan crystals. The unit cell dimensions for doped oxalic acid tryptophan crystal are compared with that of pure oxalic acid crystal and are given in **Table 1**. The molecular structures of oxalic acid and tryptophan are depicted in **Figure 2a** and **Figure 2b** respectively.

Table 1: Unit cell parameter values of pure oxalic acid and oxalic acid doped with tryptophan crystals

Cell parameters	Pure oxalic acid	Oxalic acid doped with tryptophan
a	6.114 A°	6.113 A°
b	3.587 A°	3.605 A°
c	12.011 A°	11.866 A°
α	90 $^\circ$	90 $^\circ$
β	106.13 $^\circ$	103.18 $^\circ$
γ	90 $^\circ$	90 $^\circ$
v	253.1 \AA^3	254.6 \AA^3
Crystal system	Monoclinic	Monoclinic

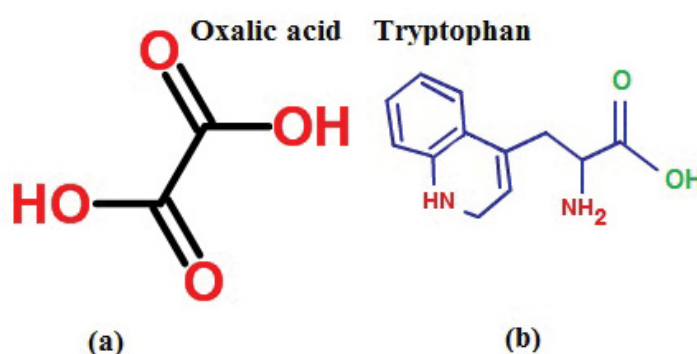


Figure 2: Molecular structures of a) pure oxalic acid and b) tryptophan.

It was observed from the single XRD measurement that pure and doped crystals belong to monoclinic system. The addition of tryptophan in oxalic acid slightly changes the crystal lattice of pure oxalic acid. This lattice distortion of pure oxalic acid confirms the incorporation of tryptophan into lattice site of oxalic acid crystal.

Powder XRD analyzes

Powder X-ray diffraction analysis was carried out to pure and doped crystals using a XPERT-PRO X-ray diffractometer with Cu K α ($\lambda=1.54060 \text{ \AA}$) radiation. It is used to identify crystalline phases and to qualitatively examine changes in crystallinity. The PXRD diffractograms of pure oxalic acid, tryptophan and the oxalic acid doped with tryptophan

crystals are shown in **Figure 3**. The d-spacing and 2θ values of oxalic acid doped with tryptophan are compared with that of pure oxalic acid crystal which is shown in **Table 2**. This study reveals that 2θ and d-spacing values of doped crystal are different from that of pure oxalic acid crystal which confirms the presence of tryptophan in the oxalic acid crystal.

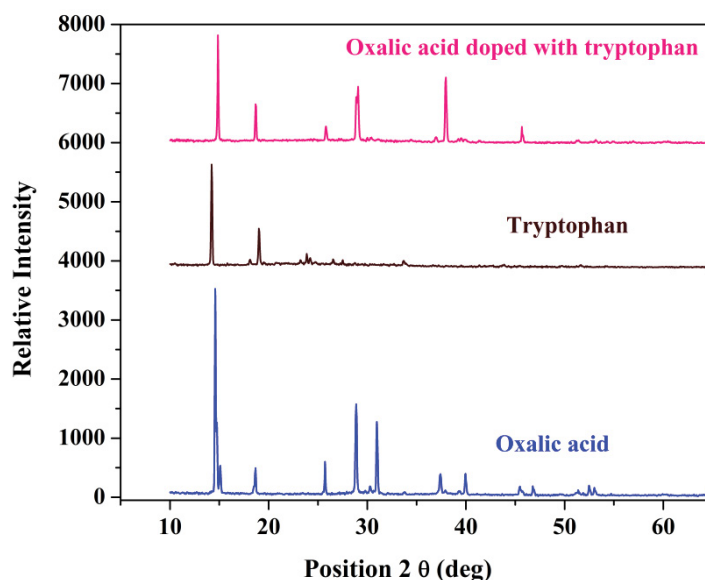


Figure 3: Powder X-ray diffraction patterns for pure oxalic acid and oxalic acid doped with tryptophan crystals.

Table 2: Powder XRD data of pure oxalic acid, pure tryptophan and oxalic acid doped with tryptophan crystals

Pure oxalic acid		Pure Tryptophan		Oxalic acid doped with tryptophan	
Position [$^{\circ}2\theta$]	d-spacing [\AA]	Position [$^{\circ}2\theta$]	d-spacing [\AA]	Position [$^{\circ}2\theta$]	d-spacing [\AA]
14.5888	6.07193	14.2329	6.22295	14.8742	5.95606
15.1026	5.86649	18.1173	4.89654	18.6935	4.74689
18.6601	4.75532	19.0364	4.66215	25.8245	3.45002
25.7377	3.46146	23.2532	3.82536	29.0711	3.07170
28.8703	3.09261	23.8829	3.72591	30.4427	2.93635
30.2872	2.95108	26.5678	3.35516	34.4203	2.60560
30.9862	2.88608	33.6879	2.66055	36.9690	2.43161
33.7763	2.65379	43.8313	2.06552	37.9731	2.36959
37.4101	2.40395	49.6522	1.83615	39.6691	2.27210
37.9522	2.37085	-	-	41.3454	2.18378
39.3222	2.29135	-	-	45.7156	1.98467
39.9675	2.25583	-	-	51.3322	1.77994
45.4587	1.99529	-	-	53.1674	1.72275
46.8252	1.94020	-	-	54.3159	1.68900
49.5899	1.83831	-	-	54.9480	1.67106
51.4138	1.77731	-	-	57.0200	1.61516
52.4920	1.74331	-	-	60.3803	1.53307
53.0482	1.72634	-	-	-	-
60.0623	1.54042	-	-	-	-
64.6839	1.44108	-	-	-	-
66.5815	1.40338	-	-	-	-
70.2638	1.33858	-	-	-	-
80.0746	1.19744	-	-	-	-

From the powdered X-ray analysis, it was confirmed that both pure and doped crystals crystallized in the monoclinic structure. Narrow peaks indicate the good crystallinity of the material. Some new peaks were observed in XRD patterns by doping with tryptophan amino acid. Due to the slight changes in intensity of some peaks were observed

as a result of doping. There is also a slight change in the lattice parameters of the doped crystals. The slight change in the intensity of peaks and lattice parameters may be due to lattice distortion by doping in the parent crystal. Hence it is revealed that dopant can enter in the lattice of oxalic acid without causing much distortion.

The crystalline size of the pure and doped crystals were determined by using the Debye-Scherrer equation, which can be written as

$$D = K\lambda / (\beta \cos\theta) \quad (1)$$

Where,

D=crystallite size

K=dimensionless shape factor (0.94)

λ =wavelength of X-ray radiation (Cu K α = 1.54060 Å)

θ =diffraction angle

β =Full width at half maximum intensity

The Dislocation density can be calculated from,

$$\delta = (1/D^2) \text{m}^{-2} \quad (2)$$

Where,

δ =dislocation density,

D=crystallite size

The average crystalline size of oxalic acid is found to be as 30 nm. But the crystalline size of the doped oxalic acid is decreased to 23 nm which confirms the presence of tryptophan in the crystal lattice of oxalic acid (**Table 3**). Also the dislocation density of oxalic acid is smaller than that of doped oxalic acid crystal.

Table 3: Average Crystalline size and Dislocation density results.

XRD parameters	Oxalic acid	Oxalic acid doped with tryptophan
Average crystalline size	30 nm	23 nm
Dislocation density	$1.12 \times 10^{15} \text{ m}^{-2}$	$1.93 \times 10^{15} \text{ m}^{-2}$

Vibrational analysis

FT-IR spectrum of the pure and doped oxalic acid crystals were carried out using SHIMADZU FT-IR spectrometer in the range 4000-400 cm^{-1} . This study is used to characterize the vibrations of various functional groups present in both crystals. The observed FT-IR spectra for pure doped crystals are given in **Figure 4**. The detailed assignments of absorption bands/peaks observed in the FT-IR spectrum of pure and doped oxalic acid crystals are shown in the **Table 4**.

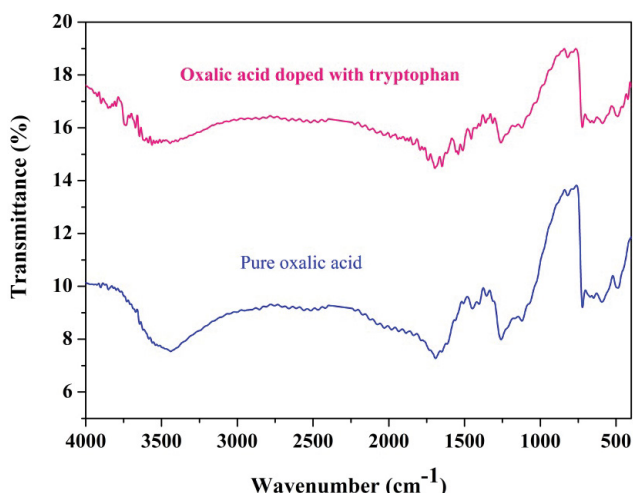


Figure 4: FT-IR spectra of pure oxalic acid and oxalic acid doped with tryptophan crystals.

Table 4: Observed wavenumbers in both spectra and their assignments for pure oxalic acid and oxalic acid doped with tryptophan crystals

Pure oxalic acid FT – IR (cm^{-1})	Oxalic acid doped with tryptophan FT – IR (cm^{-1})	Assignment
–	3563(m)	ν N-H
–	3345(m)	ν_{as} NH_2
–	3280(w)	ν_{s} NH_2
1762(s)	1742(s)	ν C=O
–	1605(sh)	ν C=C
–	1580(w)	ν C-C
–	1541(m)	δ NH_2
–	1456(w)	δ CH_2
1354(w)	1362(w)	β (OH)
–	1315(w)	ν C-N+ ω CH_2
1260(s)	1260(s)	ν C-O
–	1167(w)	ρ NH_2
–	915(sh)	ω NH_2
–	856(sh)	ρ CH_2
820(w)	820(w)	γ (OH)
–	455(w)	γ -NH

s-strong, br-broad, m-medium, w-weak, sh-shoulder, ν -stretching, ν_{s} -sym. Stretching, ρ -rocking, ν_{as} -asym. Stretching, γ -out of plane bending, β -in plane bending, δ -scissoring, ω -wagging

Vibrations of oxalic acid

The organic compound of oxalic acid has two carboxylic acid COOH groups (**Figure 2a**). The $-\text{C}(=\text{O})\text{OH}$ group is characterized by the OH stretch, the C=O stretch, C-O stretch, OH in-plane deformation and the OH out-of-plane deformation modes.

Carboxylic group vibration

The C=O stretching vibration in the spectra of carboxylic acids gives rise to strong band in the region $1725 \pm 65 \text{ cm}^{-1}$ [10,11]. In the present study, C=O stretching mode is attributed at 1762 cm^{-1} (weak) and 1742 cm^{-1} (strong) bands in IR spectrum for pure oxalic acid oxalic acid doped with tryptophan respectively. The C=O in-plane deformation is weakly to moderately active in the region $725 \pm 95 \text{ cm}^{-1}$. The bands observed at 721 cm^{-1} and 723 cm^{-1} are assigned as β (C=O) mode of pure and doped oxalic acid crystals respectively. The C=O rock has a weak intensity peak in the region $445 \pm 120 \text{ cm}^{-1}$. In the present case it is observed at 488 cm^{-1} and 490 cm^{-1} in IR spectrum of pure and doped crystals respectively. The C-O stretching of carboxylic acids appears near the region 1320 cm^{-1} to 1210 cm^{-1} in the spectra [12,13]. In the present study, the C-O stretching of carboxylic acid is identified at 1260 cm^{-1} in the IR spectrum of both compounds. The OH in-plane deformation is expected in the region $1390 \pm 55 \text{ cm}^{-1}$. The out-of-plane OH deformation exhibits a moderate band in the region $905 \pm 65 \text{ cm}^{-1}$ [14]. The band observed at 1354 cm^{-1} is assigned as β (OH) of carboxylic group of pure oxalic acid. But this mode is shifted to 1362 cm^{-1} for oxalic acid doped with tryptophan. The γ (OH) mode is identified at 820 cm^{-1} in IR spectrum of both compounds.

Vibrations of tryptophan

The aromatic amino acid of Tryptophan has an amino group, carboxyl group with one five-membered ring with a nitrogen atom bonded to a benzene ring (Indole ring, **Figure 2b**).

Amino group vibrations: The NH_2 antisymmetric stretching and symmetric stretching vibrations of primary amine occurs in the region $3365 \pm 25 \text{ cm}^{-1}$ and $3290 \pm 30 \text{ cm}^{-1}$ respectively [15]. In the vibrational spectra of oxalic acid doped with tryptophan show the absorption peaks at 3345 cm^{-1} and 3280 cm^{-1} are assigned to antisymmetric and symmetric stretching of NH_2 modes respectively. The NH_2 scissoring vibration gives rise to a broad strong band in the region $1600 \pm 50 \text{ cm}^{-1}$ [14,15]. For oxalic acid doped with tryptophan, this mode is identified at 1541 cm^{-1} in the IR spectrum. The NH_2 torsion is expected in the region $290 \pm 130 \text{ cm}^{-1}$. Rocking/twisting NH_2 mode is expected in the region $1160 \pm 140 \text{ cm}^{-1}$. In the present case, it is attributed at 1167 cm^{-1} in the IR spectrum. The wagging mode of NH_2 group show broad band at $840 \pm 55 \text{ cm}^{-1}$ in IR. For the doped crystal, the band observed at 915 cm^{-1} (IR) is assigned as ω NH_2 mode. The C-N stretching vibration of the amide group is only weakly to moderately active in the region $1385 \pm 85 \text{ cm}^{-1}$ and is difficult to detect. But this mode is downshifted to 1315 cm^{-1} in the IR spectrum of doped oxalic crystal due to the formation of hydrogen bonding.

CH₂ group vibrations: Normally, the antisymmetric stretch ($\nu_{as}CH_2$), symmetric stretch (ν_sCH_2), scissoring (δCH_2) and wagging vibration (ωCH_2) of the CH₂ group appear in the regions 3000 ± 50 , 2965 ± 30 , 1455 ± 55 and 1350 ± 85 cm, respectively [12,14]. The antisymmetric and symmetric stretching of CH₂ group bands is not observed experimentally in IR spectrum of doped oxalic acid crystal. The scissoring and wagging modes of CH₂ group are observed at 1456 cm⁻¹ and 1315 cm⁻¹ in the IR spectrum of doped oxalic crystal with tryptophan. The rocking mode of ρCH_2 is expected in the range 895 ± 85 cm⁻¹[14]. The band at 856 cm⁻¹ in the IR spectrum is assigned as ρCH_2 mode for the doped compound.

Indole ring vibrations: Absorption arising from C-H stretching mode of benzene ring occurs in the general region of 3000 cm⁻¹ to 2840 cm⁻¹ [10,11]. The CH stretching vibration is not observed experimentally. The ring carbon-carbon (C=C) stretching vibration occurs nearly in the region 1600 and 1500 cm⁻¹ and is usually stronger [14]. In the present work, the C=C modes are observed experimentally as weak bands at 1605 cm⁻¹ for doped crystal. In the case of substituted benzene, the C-C stretching mode vibrations produce the bands at 1620 cm⁻¹ to 1565 cm⁻¹ with the groups [15]. In the present work, the band at 1580 cm⁻¹ in IR spectrum is assigned to C-C stretching vibration for doped oxalic crystal only. The N-H stretching mode is observed between 3700 cm⁻¹ to 3100 cm⁻¹ in the IR spectrum [10]. This mode is observed at 3563 cm⁻¹ in the IR spectrum of doped oxalic crystal with tryptophan crystal only. The N-H out-of-plane bending is assigned to 455 cm⁻¹ in the present work of oxalic acid doped with tryptophan crystal.

Ultraviolet visible spectroscopy analyzes

The UV-Visible spectroscopy analyzes is one of the most frequently employed techniques to analyze the pharmaceutical compound. The optical transmittance and absorption spectrum of grown crystals has been recorded with SHIMADZU-UV1800, double beam spectrometer. Transmittance and absorbance data were observed for both crystals in the wavelength range 200 - 1100 nm in steps of 1 nm. The slit width chosen was 1 nm. The wavelength rate was in medium mode. The observed values of absorbance were recorded and stored in the memory of a computer and plotted. The absorbance spectrum of pure oxalic acid and oxalic acid doped with tryptophan crystals is shown in **Figure 5**.

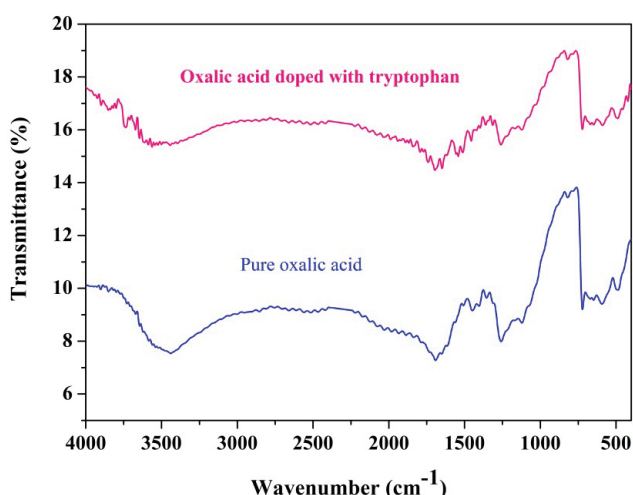


Figure 5: Absorption spectra of pure oxalic acid and oxalic acid doped with tryptophan crystals.

The maximum absorbance wavelength (λ_{max}) for pure and doped oxalic acid crystals are found to be at 214 nm, 207 nm and the lower cut-off wavelength is observed at 289 nm, 299 nm respectively. From the absorbance spectrum, the pure and doped oxalic acid crystals shows the good transmittance in the entire range of visible region.

The band gap of the crystals were estimated by using the following the relation [16].

$$E_g = (1.243 \times 10^3) / \lambda_{max} \quad (3)$$

The optical band gap was evaluated by plotting $(\alpha h\nu)^2$ vs. $h\nu$ as shown in **Figure 6** and extrapolating the linear portion of absorption edge $(\alpha h\nu)^2$ to the photon energy axis gives the optical band gap of the crystal. The band gap value of the crystal was found to be as 5 eV and 5.2 eV for pure and doped oxalic acid crystals respectively. The band gap value slightly increases while the dopant was added to pure oxalic acid. The high value of band gap indicates that the grown crystals may be act as a typical of dielectric material. It is also inferred from the spectra that both pure and doped

crystals have large transmission window in the entire visible region. The incorporation of tryptophan in the oxalic acid compound as a dopant progressively improved the optical quality of oxalic acid crystals with higher transparency.

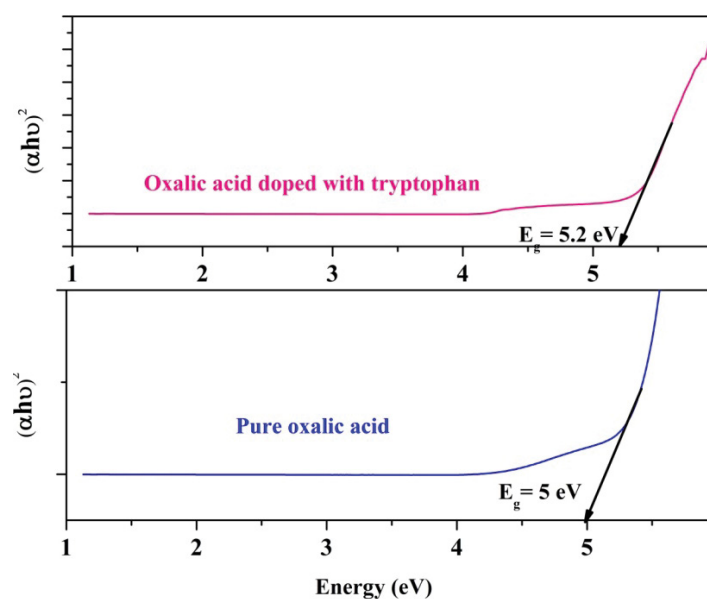


Figure 6: Variation of photon energy ($h\nu$) with $(\alpha h\nu)^2$ of pure oxalic acid and oxalic acid doped with tryptophan crystals.

Antimicrobial activity study

The antimicrobial activities of pure and doped crystals were analyzed by disk diffusion method. They are tested by two different microorganisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). Their consultancy results are shown in **Table 5**. The photographic view of zone of inhibition of both microorganisms for pure and doped crystals is shown in **Figure 7**.

Table 5: Antibacterial activity of oxalic acid and oxalic acid doped with tryptophan crystals against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacterial strains.

S. No.	Microorganisms	Zone of Inhibition in mm (50 μ l)	
		Oxalic acid	Oxalic acid doped with tryptophan
1	<i>Staphylococcus aureus</i>	12	17
2	<i>Pseudomonas aeruginosa</i>	10	11

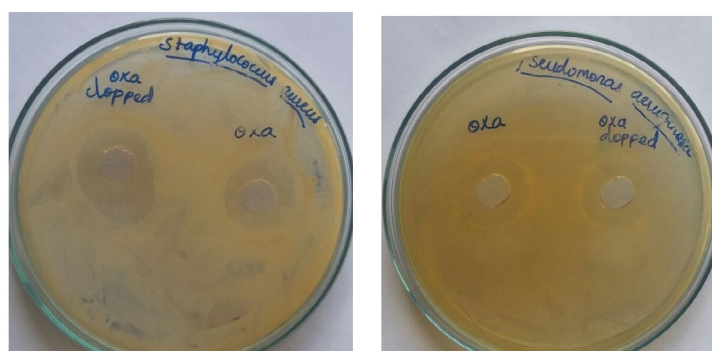


Figure 7: Photographic view of zone of inhibition of pure oxalic acid and oxalic acid doped with tryptophan crystals against the *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

This results show that doped oxalic acid crystal enhance the antimicrobial activity against the *Staphylococcus aureus* than the *Pseudomonas aeruginosa* microorganism species. Due to the drug resistance property of *Pseudomonas aeruginosa* gram negative bacteria, pure and doped crystal has almost the same diameter of zone of inhibition.

CONCLUSION

Pure oxalic acid and oxalic acid doped with tryptophan are grown by slow evaporation method. Single crystal XRD and powder XRD studies confirm that both the pure and doped oxalic acid crystals belong to monoclinic system. The data obtained by XRD analysis and evaluation of lattice parameters have confirmed that the dopant tryptophan has entered into the lattices of oxalic acid crystals. The FT-IR study confirmed the presence of functional groups of the oxalic acid and tryptophan. The UV-Vis spectral analysis reveals that the pure and doped crystals have high transparency in the entire visible region. The antimicrobial test is performed for pure and doped oxalic acid crystals against the gram positive and gram negative bacteria. This results shows that dopant of tryptophan enhance the antimicrobial activity of oxalic acid. Finally, it is concluded that the dopant crystal may be used in the pharmaceutical field for medical application.

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