

A comparison on the antibacterial activity of amino acids added Sulphamic acid crystals grown by slow evaporation method

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Abstract

The Sulphamic acid (SA) and Amino acids (L-Proline (LP), L-Valine (LV) and L-Methionine (LM)) added sulphamic acid crystals were grown by slow evaporation method at room temperature. The XRD analysis revealed that all the grown crystals possessing orthorhombic crystal structure. The Raman vibrational modes and functional group bondings present in the grown crystals confirmed the orthorhombic structure and zwitter ion formation. The presence of the constituent elements confirmed the incorporation of added elements. The optical transparency and band gap of the grown crystals were calculated. The transparency of the SA was enhanced due to the addition of L-Proline, L-Valine and L-Methionine. The strong intense blue emission due to transition from D-states to F-states find potential for blue light emitted optoelectronic applications. All the prepared crystals acted as good antibacterial agents compared with standard antibiotic AMIKACIN.

Keywords: Crystal growth, Sulphamic acid, XRD, FTIR, EDAX, SEM, optical studies, antibacterial activity.

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1. Introduction

The antibacterial activity of crystalline materials has garnered significant interest in recent years due to their potential as effective agents in combating bacterial infections. Crystals, which can range from naturally occurring minerals to synthetic compounds, possess unique structural and chemical properties that can make them highly effective in inhibiting the growth of bacteria. These materials often exhibit antibacterial properties through mechanisms such as disruption of bacterial cell membranes, interference with cellular processes, and the release of bioactive ions. The antibacterial activity of crystals is influenced by several factors, including the composition, size, and morphology of the crystals. For instance, metal-based crystals like silver, copper, and zinc have been widely studied for their antimicrobial properties. Silver nanoparticles, in particular, are known for their broad-spectrum antibacterial activity, which is attributed to their ability to release silver ions that interact with bacterial cell walls, disrupt cellular function, and generate reactive oxygen species (ROS) that cause oxidative stress. Additionally, some organic crystalline materials and pharmaceuticals in crystalline form have demonstrated antibacterial effects. For example, certain antibiotics, such as tetracycline and ampicillin, are often utilized in crystalline form and retain their antimicrobial properties when administered in this state. Crystals can also enhance the bioavailability and controlled

release of active compounds, improving their efficacy in fighting bacterial infections.

Research has increasingly focused on the synthesis and characterization of new crystalline materials with enhanced antibacterial properties. Advances in nanotechnology have also led to the development of nanocrystals with improved antimicrobial performance. These studies suggest that the precise control over crystal growth and the introduction of functional groups on the crystal surface can significantly enhance their antibacterial activity [1-4].

Crystal growth has played an important role in latest technology due to their supreme physical and chemical properties. Therefore huge research activities have been focused on the growth and characterization of pure and additive single crystals. To strengthen the properties of crystals generally dopants/additives have been added [5-9]. The inorganic crystals are familiar in the area of photonics, owing to high speed information processing, high optical data disk storage, frequency conversion and optical communication [10-15]. Amid overall inorganic crystals, sulphamic acid has got great observation because of its high thermal and chemical stability, better transmittance and good optical and nonlinear properties [16-19].

It is well known that sulphamic acid ($\text{H}_2\text{NSO}_3\text{H}$) and its derivatives get distinctive observation in NLO property due to its two planar rings which arise from the strong donor-acceptor

intermolecular interaction and delocalized π -electron configuration and having excellent blue light transmittance property [21,22]. Amid overall inorganic acids, sulphamic acid is somewhat invariable and crystallizes in non-centrosymmetric structure and hence gives good NLO property. Sulphamic acid is an odorless non-volatile solid. It is also non-hygroscopic and non-corrosive. Further it serves as a good green catalyst in organic synthesis, with outstanding piezoelectric and NLO properties. Sulphamic acid is an inorganic material having orthorhombic structure along with Pbc_a space group. It forms Zwitterions when dissolved in water and acquires well known catalytic features which prevail to remote from other acidic catalysts [11, 22-27]. Due to these advantages, the Japanese industrial committee has qualified SA as the standard substance for titrimetric analysis [28].

Owing to high thermal stability, elevated catalytic behavior and superior optical properties many research activities have been completed based on sulphamic acid with various dopants and additives. Previous studies revealed that SA was doped with various rare earth elements like lanthanum, dysprosium, terbium, neodymium, cesium, gadolinium, Yttrium and cerium and also with metallic elements copper, manganese, and nickel. Moreover sulphamic acid has been doped with compounds like LiCl, NaCl and KCl, ZnSO₄ & MnSO₄, FeSO₄, Al₂Cl₃ and thiourea [10-12, 16-18, 20, 21, 29-35]. Here, in the present work the amino acid L –proline(LP), L-valine(LV) and L-methionine(LM) added with sulphamic acid and the crystals were grown by slow evaporation method and its structural, mechanical and optical properties were investigated.

2. Experimental Methods

The crystals of pure sulphamic acid and L –proline(LP), L-valine(LV) and L-methionine(LM) added with sulphamic acid crystals were grown by slow evaporation technique using the AR grade sulphamic acid and L-Proline, L-valine and L-Methionine powders. To grow these crystals the AR grade sulphamic acid and L-Proline, L-Valine and L-Methionine powders in the molar ratio 3:1 were taken and dissolved in deionized water. The dissolved mixture was stirred well using magnetic stirrer for four hours at room temperature until well liquefied. The saturated solution was filtered using a filter paper to remove any non-miscible and unstirred chemical. The filtered transparent solution was shifted to crystal growth vessels, enclosed by aluminum foil sheet with few punched holes and stored in a dust free atmosphere. The solution was permitted to crystallization at room temperature. Good transparent and colorless crystals were collected after 20 days.

Fig. 1 shows the photograph of the grown pure sulphamic acid crystal and L-Proline, L-Valine and L-Methionine added sulphamic acid crystals.

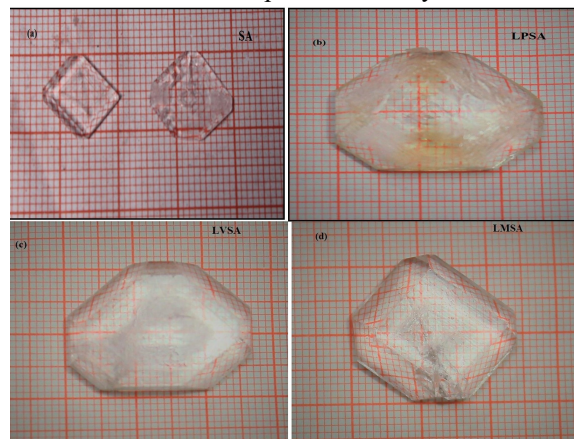


Figure 1. Photographs of the grown crystals (a) SA (b) LPSA (c) LVSA (d) LMSA

3. Results and discussions

3.1. Powder X-ray diffraction

The powder x-ray diffraction (PXRD) pattern of sulphamic acid and L-Proline, L-Valine and L-Methionine powders added sulphamic acid are shown in Fig. 2. The grown pure SA and L-Proline, L-valine and L-Methionine added SA crystals show orthorhombic crystal system. All the diffraction peaks were matched with the standard ICDD pattern (JCPDS Card No.: 70-0060). No peaks have been unindexed. The strong intensity peak (221) was obtained for pure sulphamic acid (Fig.2 (a)). Therefore many grains will be oriented in the [221] direction, (021), (210) and (211) peaks belonging to orthorhombic structure was also identified. Therefore the grown crystals were phase pure and possessed orthorhombic structure [9]. When L-Proline, L-Valine and L-Methionine powders was added with sulphamic acid, significant changes were not observed, further intensity modification was noticed. Comparing to pure SA crystal, in L-Proline, L-Valine and L-Methionine powders added crystal the intensity of intense peak (221) was decreased. Besides the intensity of (211) peak was increased. Moreover the intensity of (021) and (210) were reduced invariably. Hence the preferred grain orientation was along [211] plane. Since, no impurity peaks were identified and all the peaks were well fitted to the orthorhombic crystal structure, it was confirmed that L-Proline, L-Valine and L-Methionine powders added sulphamic acid also exhibits similar structure [30]. Using unit cell software package the unit cell parameters were calculated and tabulated. From the table 1, it was proved that the cell parameters matches well with the standard lattice parameters of sulphamic acid.

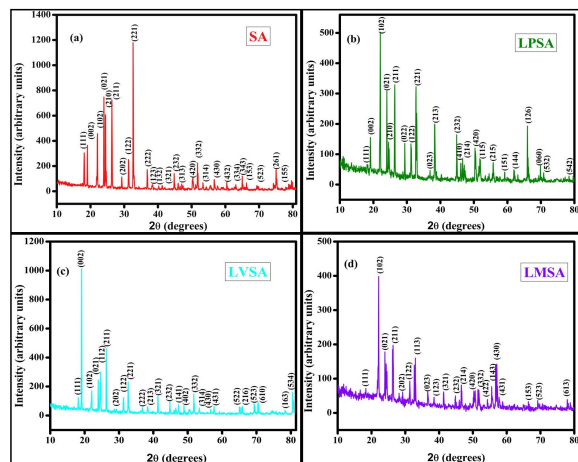


Figure 2. Powder X-ray diffraction patterns of (a) SA (b) LPSA (c) LVSA (d) LMSA crystals

Table 1. Structure and lattice parameters of pure SA and L-Proline, L-valine and L-Methionine powders crystals.

Cryst al	Structure	a(A°)	b(A°)	c(A°)	Volum e (A³)
SA	Orthorho mbic	8.12 66	8.09 28	9.22 98	607.01 66
GSA	Orthorho mbic	8.08 92	.068 7	.229 8	602.42 33
LISA	Orthorho mbic	8.12 17	8.08 33	9.24 96	607.24 34
LPAS A	Orthorho mbic	8.13 11	8.06 95	9.23 62	606.02 70

3.2. Fourier Transform Infrared Analysis

Fig. 3 shows the recorded FTIR spectra of pure SA and L-Proline, L-Valine and L-Methionine powders crystals. The FTIR spectrum of SA showed all the characteristic functional group bondings. The functional group bondings were assigned and summarized in Table 2. The IR band exhibited at 1002 cm⁻¹ was related to the rocking mode vibration of NH₃⁺ ion thus verified the zwitter ionic nature of sulphamic acid crystal [36]. The bands noticed at frequencies around 2873 cm⁻¹ and 3153 cm⁻¹ respectively were due to NH₃⁺ vibrations related to stretching as well as degenerative stretching modes. Further, the bands observed at 1447 cm⁻¹, 1806 cm⁻¹ and 1541 cm⁻¹ successively were confirmed to symmetric and degenerated modes of vibration

related to NH₃⁺ deformation. The bands observed at 1267 cm⁻¹ and 1068 cm⁻¹ were due to degenerated and symmetric SO₃⁻ stretching. The band occurred at 548 cm⁻¹ was also assigned to degenerated SO₃⁻ deformation. The N-S, N-H and S-H stretching vibrations were seen at frequencies 690 cm⁻¹, 2023 cm⁻¹ and 2458 cm⁻¹ respectively [30, 37]. All the observed IR bands were in good agreement with earlier reports and related to theoretically calculated band frequencies. Thus from the recorded FTIR spectrum of SA it was observed that all the characteristic IR bands of functional groups along with zwitter ions were observed. Hence the synthesized SA crystal was phase pure and showed orthorhombic structure. [38, 39]. Nevertheless in L-proline, L-valine and L-Methionine powders added sulphamic acid all the peaks were seen whereas the band intensities were changed.

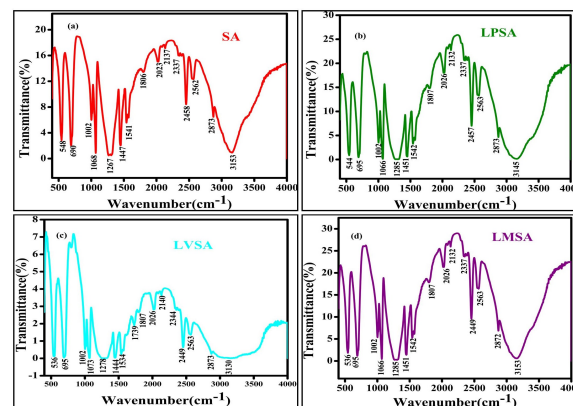


Figure 3. FTIR spectra of (a) SA (b) LPSA (c) LVSA (d) LMSA crystals

Table 2. Vibrational assignment of SA , LPSA, LVSA and LMSA crystals

Wave number(cm ⁻¹)				Assignment
SA	LPSA	LVSA	LMSA	
548	544	5	53	Degen. SO ₃ ⁻ deformation
		36	6	
690	695	6	69	N-S stretching
		95	5	
1002	1002	1	10	Rocking mode NH ₃ ⁺
		002	02	

1068	1066	1	10	Symmetric SO_3^-
		073	66	Stretching
1267	1285	1	12	Degen. SO_3^-
		278	85	stretching
1447	1451	1	14	Sym. NH_3^+
		444	51	deformation
1541	1542	1	15	Degen. NH_3^+
		534	42	deformation
1806	1807	1	18	Symmetric NH_3^+
		807	07	deformation
2023	2026	2	20	N-H Stretching
		026	26	
2458	2457	2	24	S-H Stretching
		449	49	
2873	2873	2	28	Symmetric
		873	72	NH_3^+ Stretching
3153	3145	3	31	Degen. NH_3^+
		130	38	Stretching

3.3 Raman analysis

The recorded Raman spectra of SA and L-proline, L-Valine and L-Methionine powders added sulphamic acid crystals are shown in Fig. 4. The high intensity vibration band occurred in the frequency region near 1059cm^{-1} was related to symmetric SO_3^- stretching. The vibrations appeared at 1281cm^{-1} and 1345cm^{-1} were due to degenerate SO_3^- stretching modes. The band noticed at 1017cm^{-1} was depicted to degenerated NH_3^+ rocking vibration. The N-S stretching vibration was seen at 682cm^{-1} . The vibration bands observed at 543cm^{-1} was designated to degenerated SO_3^- deformation and vibration band noticed at 363cm^{-1} was due to the presence of SO_3^- rocking vibrations[25,40-44].From the assigned

Raman modes, it was confirmed that SA and LISA crystals possess orthorhombic structure. In L-Isolucine added sulphamic acid only slight changes in peak positions were observed. The intensity of the high intense vibration band associated to degenerated SO_3^- deformation was almost retained in the same peak position. All the other peaks were slightly deviated than pure SA. Therefore it was elucidated that the addition of L-proline, L-Valine and L-Methionine powders added sulphamic acid to sulphamic acid not ever disturbed the structure of sulphamic acid, anyway small variations have been occur in the geometry of the structure.

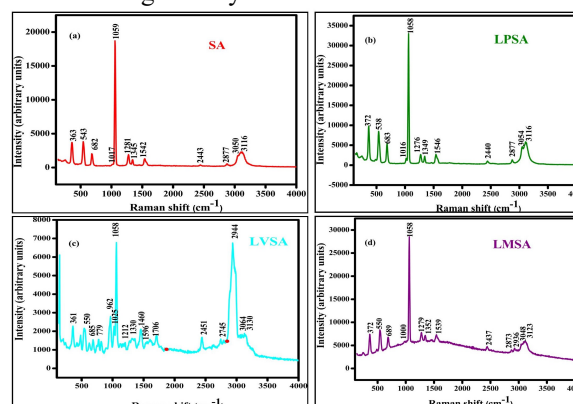


Figure 4. Raman spectra of (a) SA (b)LPSA (c) LVSA and (d) LMSA crystal

3.4 Morphology and compositional analysis

Fig. 5 shows the SEM micrographs of SA and L-proline, L-valine and L-Methionine powders added sulphamic acid crystals. From the micrographs it was observed that all the crystals have been agglomerated as clusters with voids. The agglomerated crystals were in non uniform shape and size. Due to uneven distribution of temperature while synthesis process, resulted in difference in shape and size. Similar agglomerated cluster like crystals have been observed by B. Brahmaji et al [11].

Fig. 5. Shows the EDAX spectra of SA and L-proline, L-Valine and L-Methionine powders added sulphamic acid crystals. In pure SA spectra the peaks associated to sulphur(S), nitrogen(N) and oxygen(O) were noticed.From this it was found that pure SA consists of only the constituent elements as in the formula of sulphamic acid ($\text{H}_2\text{NSO}_3\text{H}$). But in L-proline, L-Valine and L-Methionine powders added sulphamic acid added sulphamic acid the peaks related to the elements S, N, O were seen. Additionally the peak affirmed to carbon (C) was also appeared. Hence it was realized that the added L-proline, L-Valine and L-Methionine powders added sulphamic acid was incorporated in to the SA lattice. The elemental composition of SA , LPSA, LVSA and LMSA crystals were tabulated in table 3a, 3b, 3c and 3d respectively.

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Table 3a. Elemental composition of pure SA

Element	Atomic weight (%)	Molecular weight (%)
Sulphur	12.37	22.51
Oxygen	69.71	63.26
Nitrogen	17.91	14.23

Table 3b. Elemental composition of LPSA

Element	Atomic weight (%)	Molecular weight (%)
Sulphur	19.95	34.61
Oxygen	52.48	45.42
Nitrogen	19.08	14.45
Carbon	8.48	5.51

Table 3c. Elemental composition of LVSA

Element	Atomic weight (%)	Molecular weight (%)
Sulphur	19.95	34.61
Oxygen	52.48	45.42
Nitrogen	19.08	14.45
Carbon	8.48	5.51

Table 3d. Elemental composition of LMSA

Element	Atomic weight (%)	Molecular weight (%)
Sulphur	19.95	34.61
Oxygen	52.48	45.42
Nitrogen	19.08	14.45
Carbon	8.48	5.51

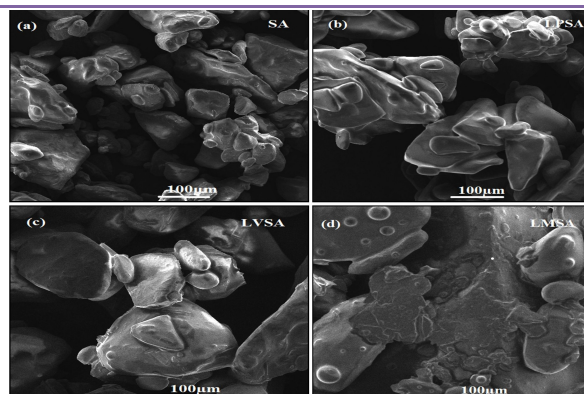


Fig. 5a SEM analysis of (a) SA (b) LPSA (c) LVSA and (d) LMSA crystal

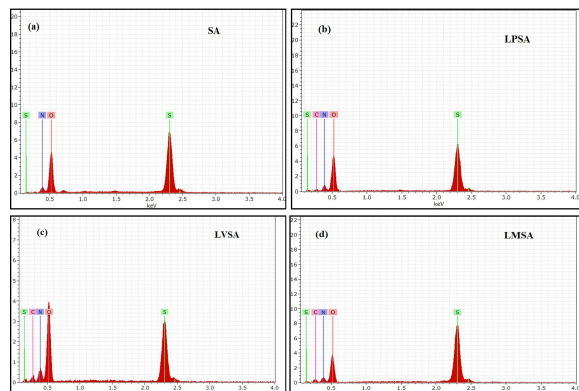


Fig. 5b EDAX analysis of (a) SA (b) LPSA (c) LVSA and (d) LMSA crystal

3.5. UV-VIS studies

UV-VIS spectral analysis was carried out in the wavelength range 200nm to 800nm to find the absorbance and transmittance of the prepared crystals. The transmittance graph (Fig. 6) predicted that the prepared SA and L-Proline, L-Valine and L-Methionine powders added sulphamic acid crystals possessed a wide range of transmission window above 360nm similar to previously reported results [45]. The percentage of transmittance was 86 for pure SA whereas for L-Proline, L-Valine and L-Methionine powders added sulphamic acid the transmittance percentage was 91. This increase in transmittance makes this material useful for photoluminescence applications. From the absorbance graph (Fig. 7) it was absorbed that the lower cut-off wavelength was about 225nm for SA crystal L-proline, L-valine and L-Methionine powders added sulphamic acid [46].

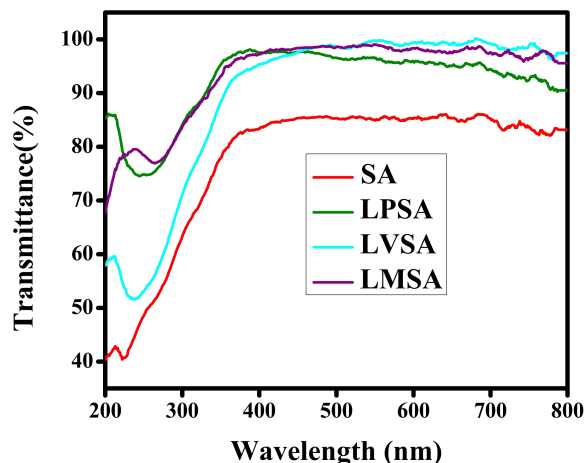


Figure 6. UV Transmission spectra of (a) SA (b) LPSA (c) LVSA and (d) LMSA crystals

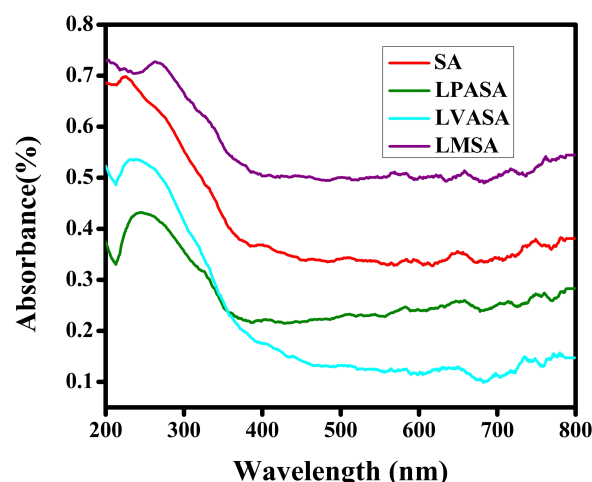


Figure 7. UV Absorption spectra of (a) SA (b)LPSA (c) LVSA and (d) LMSA crystal

3.6 Antibacterial activity

The antibacterial activity of SA and amino acids added SA was investigated against gram positive bacteria (*Bacillus*, *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*, *Klebsiella*, *Serratia*) by Disk Diffusion method and Kirby – Bauer method. Reagents used for the Disk Diffusion Test are Mueller-Hinton Agar medium. For known standard antibiotic, the zone of inhibition for *E. coli* has 22mm of half life. The present study revealed that for the pure and amino acid added SA, the zone of inhibition is high. Among all, for L-proline, L-valine and L-Methionine powders added sulphamic acid the zone of inhibition was increased up to 35. This observation confirms that LMSA sample has high power in killing bacteria. Similarly in *Klebsiella*, *Bacillus*, *Staphylococcus aureus* and *Serratia*, the absorbed zone of inhibition was increased when compared with standard antibiotic zone of inhibition. Table 4 shows the antibacterial activity of SA and amino acids added SA. From this we concluded that the grown crystals are more effective than standard antibiotic inhibition. Hence it was found that the grown crystals can be used in detergents for killing bacteria [47].

Table 4. Antibacterial effect of pure SA and amino acids added SA crystal.

Bacteria name	SA Zone of inhibition [mm]	LPSA Zone of inhibition [mm]	LVS A Zone of inhibition [mm]	LMS A Zone of inhibition [mm]	Standard antibiotic AMIKACIN Zone of inhi

					bitio n[mm]
E.C OLI	28	32	32	35	22
KL EBSIEL LA	30	31	31	30	19
BA CILLUS	29	33	33	32	20
STA PHYLO COCCU S AUREU S	26	31	31	35	22
SER ATTIA	33	33	33	32	19

3.7 Conclusion

SA and amino acids added SA single crystals were grown successfully by slow evaporation method at room temperature. Powder X-ray diffraction studies confirmed the crystalline nature and orthorhombic structure of the synthesized crystals. FTIR and FT-RAMAN analysis revealed the stretching and deformation vibrational modes of NH_3^+ and SO_3^- . The morphological and compositional analysis proved that, on addition of amino acids in SA, the crystallinity was improved and the presence of carbon was identified. The optical study showed that the crystalline perfection was excellent and the transparency was enhanced upon addition of amino acids. The antibacterial activity

showed that upon addition of amino acid the antibacterial activity was enhanced. But L-methionine added sulphamic acid (LMSA) showed a higher antibacterial activity than other amino acids which will be helpful for killing pathogenic bacteria's.

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