CHEMICAL MODIFICATION OF SOLANUM XANTHOCARPUM LEAVES RUBISCO DUE TO ACIDIC STREES

Ashwini A. Pawar[1], Anita S. Goswami-Giri[2]*

Article History: Received: 28.02.2023 **Revised:** 12.04.2023 **Accepted:** 27.05.2023

Abstract

Rubisco is a major limitation of photosynthetic CO_2 assimilation in C_3 plants. Crop yields depend on the efficiency of carbon fixation through Rubisco. The inefficiency of Rubisco by reacting with oxygen to carry oxygenation reaction instead of carboxylation reaction. The low catalytic efficiency results of Rubisco provides strong motivation to reengineer the enzyme with the goal of increasing crop yields. Hence, to established synergic link between various acids and Rubisco was evaluated by using wasteland weed solanum xanthocarpum. Modified Rubisco activity with various acid and evaluated in relation to Km, Vmax, chlorophyll content, UV, and FTIR.

Km s directly proportional to the concentration of acid. Disappearance and shifting of the band were observed in UV and FTIR. Rubisco content (0.66 to 0.96 mg/ml) , Total protein (12%), total carbohydrates (18%), Total chlorophyll a/b ratio 0.88 were calculated from wet Solanum xanthocarpum leaves. The Effect of acids exhibiting the augmentation of Photosynthesis/foodstuff quality due to the chemical modification of Rubisco.

Keyworkds: amino acids; kinetics; Photorespiration**;** Photosynthesis**.**

[1,2*]Chemistry Research Laboratory, Department of Chemistry, B. N. Bandodkar College of Science, Thane- 400601 (Maharashtra), India.

Corresponding Author:
Anita S. Goswami-Giri[2]*
[2*]Chemistry Research Laboratory, Department of Chemistry, B. N. Bandodkar College of Science, Thane- 400601 (Maharashtra), India.
Email: [2*]anitagoswami@yahoo.com

DOI: 10.31838/ecb/2023.12.s3.423

1. INTRODUCTION

Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) (EC 4.1.1.39) being one of the slowest and one of the largest enzymes in nature, with a molecular mass of 560 kDa. IT catalyzes two main reactions, Carbon fixation in photosynthesis to converts solar energy into biomass and photorespiration[1].

In photosysnthesis, owing to the wasteful oxygenase activity and slow turnover of Rubisco, is responcible to improve the photosynthetic efficiency of vascular plants. The complex nature of Rubisco's assembly has made manipulation of the enzyme extremely challenging[2]. Since Rubisco has been converting CO_2 into organic carbon to the extent that earth's current atmosphere is rich with oxygen. Nevertheless, the inefficient enzyme has faced additional exertion that it's begun to incomprehensible its natural diet due to carbon dioxide molecules with oxygen, that's deviant for photosynthesis. According to researchers, when Rubisco commands oxygen, around 20 percent of the time, it forces the plant to undergo an energy-consuming process which is known as photorespiration. During photorespiration, enzymes propel through three different compartments such as stroma, the fluid-filled area of a chloroplast outside the thylakoid membranes within the plant cell[3].

Magnesium ion of Rubisco is surrounded by small sugar molecule, three amino acids, that are Lysine, Aspartic acid, Glutamic acid (Lys^{201}, Asp^{202}, Asp^{203}, Glu^{204}) and CO_2 molecules. The small sugar molecule is similar to the product produced in the Calvin Cycle. An activator, CO_2 molecule of lysine responsible for enzyme on-off mechanism in plants. During daytime, the enzyme is turned on and at night-time, the enzyme is turned off. When the enzyme is turned on, the magnesium binds to Ribulose Bisphosphate by attaching to two oxygen atoms and the carbon dioxide molecule that is connected to the sugar[4,5].

Since all biomass results from the act of Rubisco in photosynthesis, increasing crop yields ultimately depends on improving the efficiency of carbon fixation[6]. The low catalytic efficiency of Rubisco provides strong motivation to reengineer the enzyme with the goal of increasing crop yields[7]. The activity of Rubisco and Rubisco activase, which decreased due to denaturing agents, did not demonstrate significant improvement when compared to the control[8]. Stress-related signaling compounds may directly or indirectly affect various physiological processes, including photosynthesis[9]. An abundant protein, Rubisco is concentrated in leaves responsible for food. *Solanum Xanthocarpum* **(**Solanaceae**)** is having numerous pharmaceutical properties[10]**.** Hence, present papers paid attention on consequence of Acids on Rubisco from *Solanum Xanthocarpum* Leaves.

2. MATERIAL AND METHOD

Material:
Tris buffer, $NaHCO_3$, Magnesium chloride, Mercaptoethanol, EDTA, Ascorbic Acid, KOH, Polyethylene glycol (P-4000), $HClO_4$, SDS, Glycerol, Oxalic Acid, Cinnamic Acid, Tartaric Acid, Ascorbic Acid, Succinic Acid and Sulphanilic Acid were procured from SD Fine Chemicals, Mumbai and ATP, NADPH, Dithiothreitol, Ribulose 1,5- bisphosphate (RuBP), GPDH, PGK from Sigma Aldrich. All chemicals and reagents were used of analytical grade.

Source:
The *Solanum xanthocarpum* leaves for isolation of Rubisco are collected from Ambernath, District-Thane, Maharashtra state, India in May 2018.

Methods:
Proper evaluation of Rubisco properties in relation to effect of acid, it is necessary to isolate the enzyme in pure form.

Isolation of Rubisco:
Solanum xanthocarpum leaves (250 Kg) kept in darkness overnight was frozen in liquid nitrogen and pulverized in a mortar and pestle. The powder was mixed with 25ml of 50mM Tris (pH8.0), 20mM $NaHCO_3$, 60mM $MgCl_2$**,** 5mM ascorbic acid**,** 10mM Mercaptoethanol, 1mM EDTA and then homogenized using homogenizer; the homogenate was filtered and centrifuged. Rubisco was precipitated by adding 60% (w/v) polyethylene glycol to yield a final concentration of 18%. This solution was kept at 0^oC for 1 hr. with stirring and centrifuged at 12,000 rpm for 30 min. The precipitated Rubisco with bound inhibitor was dissolved in minimum quantity of water. $HClO_4$ (0.45M) was added to precipitate Rubisco to release Rubisco from the bound inhibitor (Ca-1P).
Isolated Rubisco was isolated with salt and solvent method and compared with the Standard Rubisco and further detailed investigation was done with UV spectrophotometer and FTIR.

Activity assays of Rubisco:
Rubisco activity was measured essentially as described by Edmondson et al.(1990) with slight modification. This is a coupled or linked assay, in which the immediate product of Rubisco, 3-phosphoglycerate, is converted primarily into 1,3-bisphosphoglycerate, and then to glyceraldehyde 3-phosphate using ATP, NADPH and glycolytic enzymes such as phosphoglycerokinase and glyceraldehyde 3-phosphate dehydrogenase. The disappearance of NADPH was monitored by the $\mathbf{A_{340}}$**.**
The assay was initiated in a total volume of 0.75 ml. In a cuvette, 0.375ml buffer, sample and enough H_2O

followed by 75 µl NADH, and 50 µl coupling enzymes was added and mixed thoroughly. Finally 150µl RuBP mixed quickly and effectively and measured by spectrophotometer (Shimadzu UV-1800).

Determination of Protein

Protein Concentration was determined by the Lowry method using casin as standard [11] .

Measurement of Rubisco content:

Rubisco content was measured at 280 nm and calculated by the following equation according to Wishnick and Lane (1971)[12].

Content (mg/ml) = A_{280} × 0.61.

Analysis Of Chlorophyll Content:

Solanum xanthocarpum (2 g) was grounded 80% acetone. The acetone extracts were read at 645 and 663 nm for determination of chlorophyll *a* and chlorophyll *b* on a double beam UV-1800 spectrophotometer (UV-160A, Shimadzu Scientific Instruments, Kyoto, Japan) by the method of Arnon (1949)[13].

Effect of various Acid Components on Rubisco activity :

Concentration (0.1 M) of Oxalic Acid, Cinnamic Acid, Tartaric Acid, Ascorbic Acid, Succinic Acid and Sulphanilic Acid incubated to the *Solanum xanthocarpum* Rubisco with varied concentration ranging from (10 µL to 100 µL) separately. The reaction activity is measure as per the assay mentioned above at A_{340}. Effect of various acid on *Solanum xanthocarpum* Rubisco was measured by plotting Rubisco concentration versus activity, by keeping *Solanum xanthocarpum* Rubisco concentration as a control. Assays were initiated with activation and deactivation of enzyme in triplicate.

Determination of Km and Vmax for various actions on Rubisco activity:

The kinetic parameters $V_{max\ and}$ K_m were determined according to Michaelis-Menten equation.

UV Analysis Of Rubisco And Treated With Various Acids.-

UV analysis of pure Rubisco (50µl) and various acids (10µL) treated Rubisco effectively measured by using spectrophotometer (Shimadzu UV-1800).

FTIR Analysis Of Rubisco And Treated With Various Acids.-

The isolated *Solanum xanthocarpum leaves* Rubisco was treated with acids the change in molecular reaction was observed through FT-IR spectroscopy. The data were compared with without treated *Solanum xanthocarpum leaves* Rubisco. FT-IR data were recorded at an
absorbance mode of 4000 – 600 cm-1 wavenumber.

3. RESULT AND DISCUSSION

An abundant protein, Rubisco is the key enzyme in the photosynthesis which carries carboxylation to convert inorganic carbon into organic carbon. However, it also shows inefficiency by reacting with oxygen to carry oxygenation reaction.Therefore it reduces photosynthetic efficiency. The changes in Rubisco's activity is due to Rubisco forms the bond with the acids. Obviously, the mode of action is diverted which is depends on various factors including environmental conditions, concentration, temperature etc.

The isolated *Solanum xanthocarpum* l.Rubisco is light dependant and showing multiple mechanism. Bathellier C. (2018) explained the link between CO_2 and O_2 reactivity considering both the nature and the complexity of the chemical reaction.[14] Rubisco's catalytic activity by comparison with other chemically related enzymes is need to improving its catalytic efficiency. Rubisco was isolated and purified according to the procedure of Edmondson et al from *Solanum xanthocarpum l*.[15]. Rubisco kinetics was intensely studied by researchers still need to understand the catalytic mechanism with various acids.

Higher activation level of *Solanum xanthocarpum* leaves Rubisco was recorded at 20µl of enzyme concentration. Its activity was gradually decreases but it was very negligible up to 80µl of enzyme. 20µl of enzyme was selected for further study.

Total protein (12%) and total carbohydrates (18%) were calculated from wet *Solanum xanthocarpum* leaves. Rubisco (5 cm3) contains 2412 IU activity.

Effect of various Acids on Rubisco activity :

It was interested to estabilished synergic link between acids and Rubisco, the change in the pH of solution largely affects on the Rubisco activity. The change in the activity of Rubisco was was not recovered with the salts treatment. The soluble and insoluble acids treated with pure Rubisco**.** The km and Vmax of acid treated Rubisco were calculated by michalis-menten equation and was compared with the without treated Rubisco shown in table 1. The change in the pH of solution largely affects on the Rubisco activity.The change in Rubisco activity is shown in figure 1.

An organic acid, oxalic acid (OA) is distributed widely in plants, fungi and animals and plays different roles in different living organisms[16]. OA treatment enhanced the abundances of three chloroplastic proteins Rubisco[17] are actively degraded and are hardly synthesized again during leaf senescence suggesting that exogenous OA treatment inhibited chloroplast degradation ultimately effect on Rubisco. The figure 1 exhbited increasing in specificity of CO2/O2 due to oxalic acid. Increase in concentration

of oxalic acid was treated to rubisco (10µl) showed the removal of more insealed protein.

Cinnamic acid an aromatic carboxylic acids appearing naturally in the plant and acts as antimicrobial agents. It often appear as ester conjugates with quinic acid/chlorogenic acids. It is form through biosynthetic pathways since *Solanum Xanthocarpum* contains acids, esters, sugars , lipids, or form with aromatic amides and aliphatic amines. Rubisco fixes the CO_2 molecule from the sugar molecules in the plant[18]. Due to chemical composion of *solanum xanthocarpum*, it have medicinal values; which may change its potency, permeability, solubility or other parameters of a selected drug or pharmacophore. Due to assimilation of Rubisco, its amino acids (Lys[201], Asp[202], Asp[203], Glu[204]) varies the structure. Cinnamic acid and oxalic acid showed the exhibition of Rubisco content more than one when treated with (70µl) of concentration while tartaric acid showed effect at (80µl) (Table 2).

Enzymatic on-off mechanism was observed, when isolated Rubisco was incubated with various concentration of Tartaric acid. The formed CO_2 from tartaric acid inhibits the decarboxylation (Figure 1) It may be due to chelation of Mg^{2+} ion. Nevertheless, Rubisco activity was frozen in refrigerator and measured periodically to observed the considearable lost in activity (Table 2).

The most abundant plants antioxidant, ascorbic acid (Asc) serves as a major contributor to the cell redox state. Exposure to environmental ozone may cause significant damage to plants by imposing conditions of oxidative stress. The effect of ascorbic acid on *solanum xanthocarpum* leaves Rubisco examined whether increasing the level of ASC through enhanced Asc recycling may limit the deleterious effects of environmental oxidative stress[19].

The use of succinic acid in augmenting or enhancing the taste of foodstuffs is disclosed by (Tonsbeek in U.S. Pat. No. 3,615,600)[20] wherein an artificial flavoring mix ture is described which imparted a meaty flavor to foods, and the mixtures contain an amino acid including glutamic acid, a nucleotide and critical amounts of succinic acid and a hydroxy carboxylic acid including lactic acid.

Succinic acid increases taste and the organoleptic properties of foodstuffs or flavor in food. It is use in augmenting or enhancing the taste of foodstuffs is disclosed by Tonsbeek[21] and suggested only sulphur bearing amino acids and salt, nucleotide provides effect on food stuff including tingling and brightness along the sides of the tongue with a slight metallic astringent after taste. It showed average Rubisco content from 0.66 to 0.96. Tartaric Acid exhibited little or no affect, slight sourness or tastiness with astringency[22].

Changes of scavenger enzymes activitywa sobserved due to Sulphanilic Acid treated with rubisco. photosynthesis process is in leaves where cholorphyll affected due to various ions. However, cholorphyll degradation reduced the capacity of photosynthesis. Chlorophyll content shown in Table 1.

Determination of Km and Vmax

Vmax is same in case of oxalic acid, cinnamic acid ,ascorbic acid, siccininc acid and sulphanilic acid. Standard Rubisco content observed higher when 100 ul of oxalic acid incubated with Rubisco while it was constant from 80 ul to 100 ul when Rubisco was incubated with Tartaric Acid (Table 3).

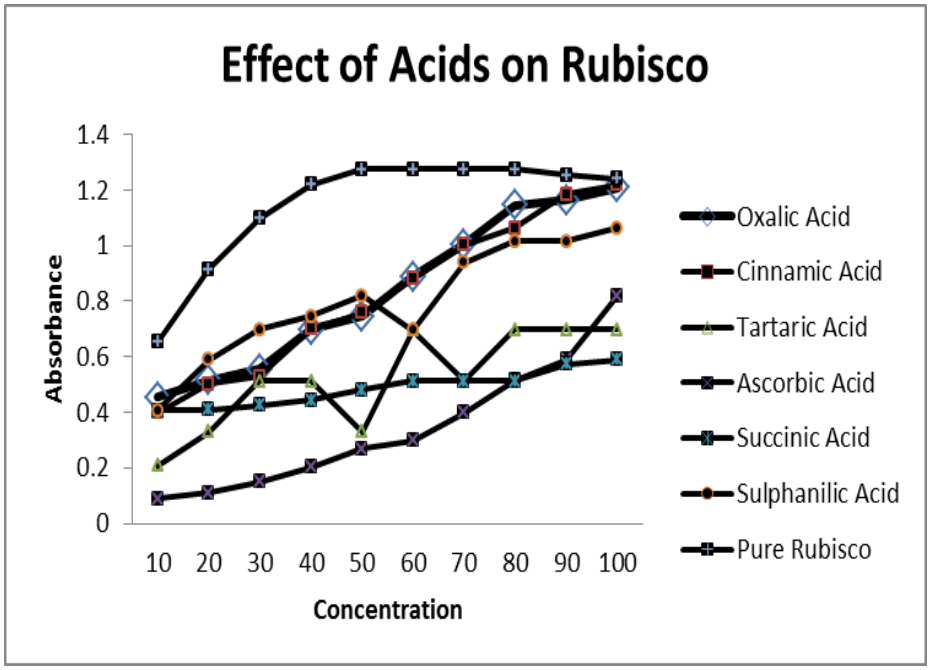

Figure 1 Effect of various acids on Rubisco

Compounds	Km	Vmax
Rubisco	**1.25**	**50**
Oxalic Acid	**1.2**	**100**
Cinnamic Acid	**1.2**	**100**
Tartaric Acid	**0.7**	**80**
Ascorbic Acid	**0.8**	**100**
Succinic Acid	**0.6**	**100**
Sulphanilic Acid	**1.05**	**100**

Table 1: KM and Vmax values of Various acids with Rubisco

Sample	Wavelength (nm)	Absorbance	g/lit	Total Chlorophyll	a/b Ratio
Chlorophyll- a	663	0.448	4.7992×10^{-3}	10.28×10^{-3}	0.8898
Chlorophyll- b	645	0.331	5.3937×10^{-3}		
	480	0.466			

Table 2 Cholorophyll content a and b observed in *Solanum xanthocarpum leaves* Rubisco.

Concentration of various acid (µl)	Change in Content of Rubisco on Acid treatment						Standard Rubisco content
	Oxalic acid	Cinnamic acid	Tartaric acid	Ascorbic acid	Succinic acid	Sulphanilic acid	
10	0.45506	0.39833	0.20862	0.08784	0.40687	0.40687	0.65392
20	0.51545	0.50081	0.3294	0.10919	0.40931	0.58865	0.915
30	0.55632	0.52887	0.5124	0.14884	0.42639	0.69723	1.098
40	0.69906	0.69967	0.5124	0.20313	0.44347	0.74542	1.22
50	0.74542	0.75884	0.3294	0.27023	0.4819	0.81862	1.27612
60	0.88877	0.88023	0.69662	0.29768	0.51362	0.69662	1.27551
70	1.00406	1.00284	0.5124	0.39833	0.51362	0.94001	1.27551
80	1.14558	1.06323	0.69723	0.51484	0.51362	1.01565	1.27612
90	1.1651	1.18462	0.69723	0.58987	0.57401	1.01565	1.25477
100	1.20963	1.22	0.69723	0.81923	0.58865	1.06323	1.24135

Table 3 *Solanum xanthocarpum leaves* Rubisco Content with various acids are calculated by Wishnick and Lane (1971)[12].

UV Analysis Of Rubisco And Treated With Various Acids.-

UV spectra for the activity of Rubisco on exhibited disappearance of NADPH was monitored at A_{340} nm is shown in figure 2. The isolated pure Rubisco (50µl) from solanum xanthocarpum leaves and effect of various acid (10µL)such as ascorbic acid , cinnamic acid , Oxalic acid, Sulphanilic acid, Succinic acid,Tartaric acid treated Rubisco and measured the activity effectively by spectrophotometer (Shimadzu UV-1800).shown in figure 3. All exhibited maxima with various acids shifted to the higher wavelength. Prompted by observations that the yield of activity appeared to differ between rubiscos from different concentration of acids for catalytic ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco).

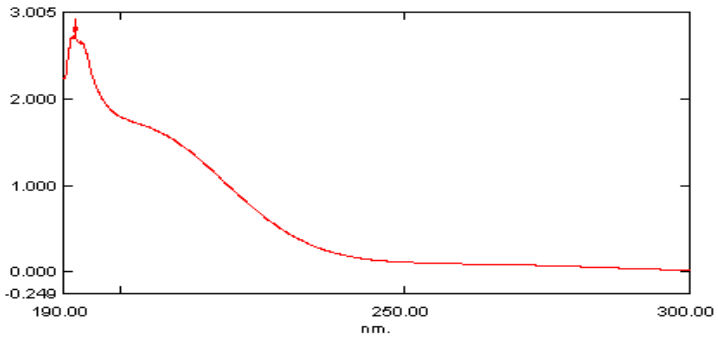

Figure 2 The activity of Rubisco on UV spectrophotometer shows disappearance of NADPH.

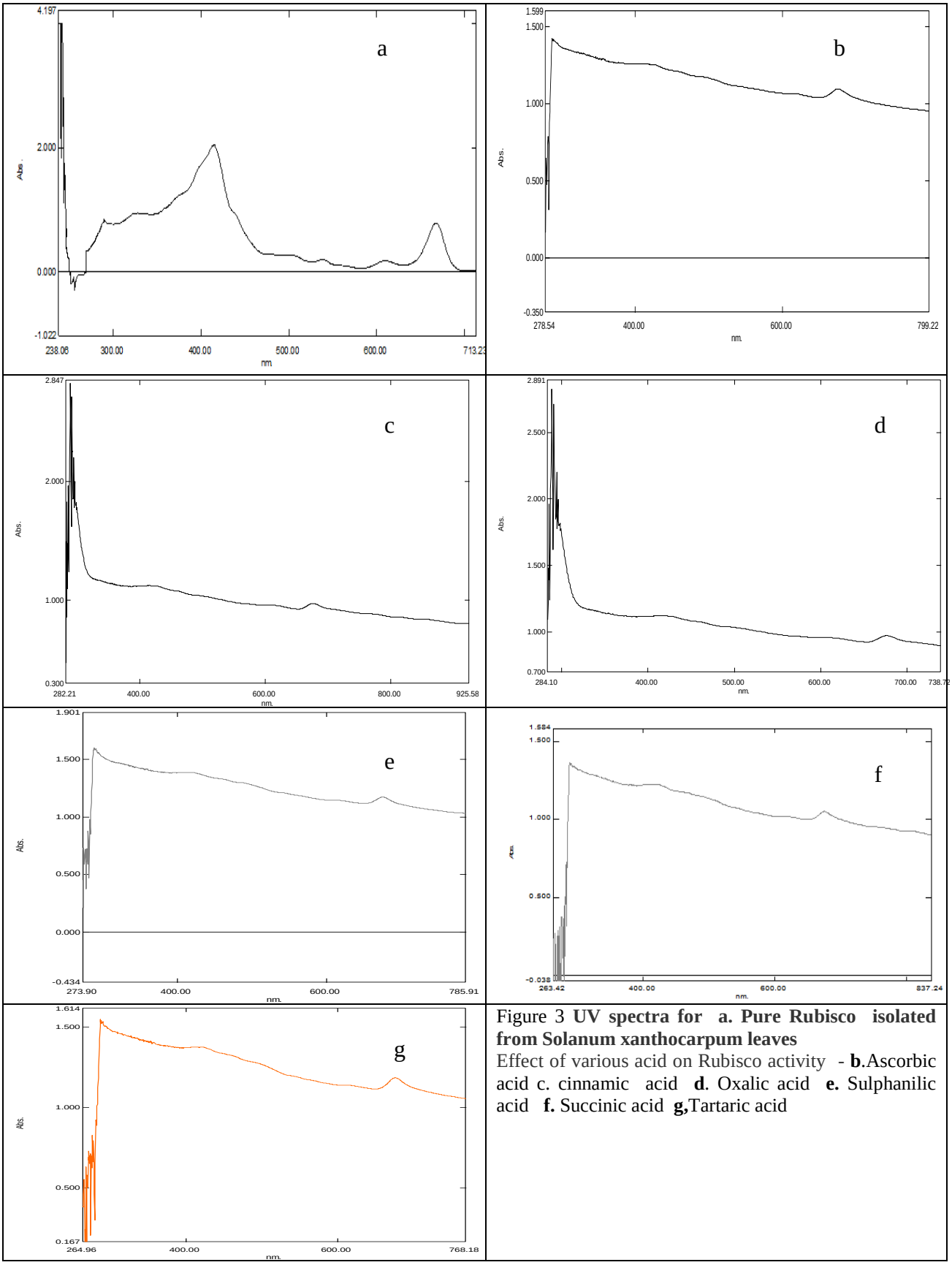

Figure 3 **UV spectra for a. Pure Rubisco isolated from Solanum xanthocarpum leaves**
Effect of various acid on Rubisco activity - **b**.Ascorbic acid c. cinnamic acid **d**. Oxalic acid **e.** Sulphanilic acid **f.** Succinic acid **g,**Tartaric acid

FTIR Analysis of various Acids bounded Solanum Xanthocarpum l. Rubisco

FTIR is powerful tool for the molecular analysis of reaction. The absorption bands from the spectra in this

study suggest that the the amide groups are rich components of proteins mostly containing N-H structures. FTIR is a nondestructive analytical technique that is less time consuming and cheaper providing unique molecular chemical information based on the FTIR spectrum. The FTIR of pure Rubisco is shown in figure 4.

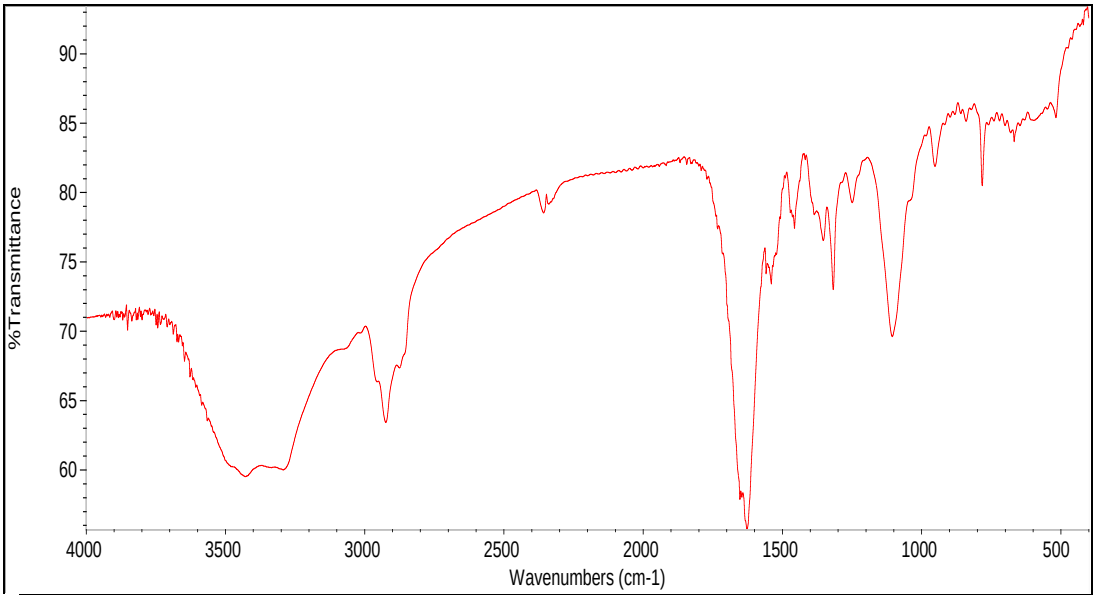

Figure 4 FTIR spectra Pure Rubisco isolated from Solanum xanthocarpum leaves

Table 4 FTIR Analysis of various Acids bounded Solanum Xanthocarpum l. Rubisco.

Various Acids with Rubisco	FTIR Analysis
Pure rubisco	3300-3600 cm^{-1} Monomeric forms: sharp O-H (alcohol). , 2900-3000 cm-1 (sp3) alkyl C-H bands , 2350 cm-1 acquisition has a possibility to compensation CO2 and water,1620 cm-1, 1450 cm-1 (C-C stretch (in ring), 1100cm-1 (C-N stretching), 950m-1 (O-H) bending out of plane with the vibration of =C-H bend,795cm-1aromatic monosubstituted
Oxalic Acid	3300-3600 cm^{-1} Monomeric forms: sharp O-H (alcohol). H-bonding leads to broadening, 2800-3000 cm-1 (sp3) alkyl C-H bands,1600-1800 cm-1 C=O, Acids: 1650-1700 cm-1, Ketones: 1720-1750 cm-1, P=O stretch 1320-1140 cm-1 strong, C-O 1200-1300 cm-1, 995-885 cm-1 Alkene strong bending at C=C, O=P-OH broad IR bands involving OH stretch from 2725-1600 cm-1 maxima at 2725-2525 cm-1 , 2350-2080 cm-1 , and 1740-1600 cm-1 –OH group Peak at 1740-1600 cm-1 is strongest P-O stretch at 1040-909 cm-1, Out-of-phase P-O-C stretch 1088-920 cm-1 strong in IR, P=O stretch 1320-1140 cm-1 strong. O=P-OH broad IR bands involving OH stretch from 2725-1600 cm-1
Cinnamic Acid	3700-3600 cm^{-1} medium sharp stretching O-H (alcohol), strong broad stretching C=O Acid: 1650-1700 cm-1, 1200-1300 cm-1 C-O ,C=C 1680-1620 cm-1 Alkene, Variable Benzene Ring 1600 cm-1 and 1500 cm-1 often has 2 peaks, C=C 995-885 cm-1 Alkene strong bending (monosubstituted or |disubstituted), O=P-OH broad IR bands involving OH stretch from 2725-1600 cm-1,–OH group Peak at 1740-1600 cm-1 is strongest P-O stretch at 1040-909 cm-1, P-O-C stretch 1088-920 cm-1 strong in IR, strong. O=P-OH broad IR bands involving OH stretch from 2725-1600 cm-1
Tartaric Acid	2500-3600 cm^{-1} H-bonding leads to broadening. O-H (alcohol)O-H (carboxylic acid), C-O 1200-1300 cm-1 , broad peak at 1740.33 cm-1 saturated C = O stretching, P-O stretch at 1040-909 cm-1, P-O-C stretch 1088-920 cm-1 strong.
Ascorbic Acid	strong bands at 3526 cm-1, 3411 cm-1 and 3317 cm-1 O-H, stretching C-H 2800-3000 cm-1(2807 cm-1 C-H asym. stretching in CH2 group), 1860 cm^{-1} C=O stretching vibration, 1754 cm^{-1} and 1674 cm^{-1}C-O stretching, O=P-OH broad IR bands involving OH stretch from 2725-1600 cm-1 ,C=C 995-885 cm-1 Alkene strong bending, 1321 cm^{-1} and 1275 cm^{-1} C-OH bands, P-O stretch at 1040-909 cm-1, P-O-C stretch 1088-920 cm-1 strong.
Succinic Acid	O-H 3650-3200 cm^{-1},C-H 2800-3000 cm-1 (sp3) alkyl C-H bands,C=O 1732 cm-1, C-H (Alkane) 1465-1375 cm-1, medium bending C-O 1210-1163 cm-1, P=O stretch 1320-1140 cm-1 strong

Sulphanilic Acid	3300-2500 cm^{-1} strong, broad, starching of O-H (carboxylic acid), C-H (aromatic compounds) 2000-1650 cm-1 bending, C=O Acids 1650-1700 cm-1, Amides:1650-1715 cm-1, S=O (sulfate) 1415-1380 and 1200-1185 cm-1 stretching C-O 1200-1300 cm-1 ,C=C 995-885 cm-1 Alkene strong bending, P-O stretch at 1040-909 cm-1, Out-of-phase P-O-C stretch 1088-920 cm-1 strong in IR, P=O stretch 1320-1140 cm-1 strong

FTIR spectra within the wave number ranges of 3050 cm−1 to 2800 cm−1, 1750 cm−1 to 1250 cm−1 and 1250 cm−1 to 900 cm-1 vibration of the peptide bond containing C=O (80%) and N–H stretching (20%), appearing between 1700 cm−1 and 1600 cm−1 provided information about protein[19]. The presence of α-hydroxyl groups in reducing sugars was found to shift the stretching frequencies of sugar carbonyl bands to higher values relative to simple alkyl-substituted carbonyl compounds.

The intensities: 3050 cm−1 to 2800 cm−1,1750 cm−1 to 1250 cm−1 and 1250 cm−1 to 900 cm-1 bands corresponding to lipids, amides and carbohydrates, respectively, These changes associated with proteins, Rubisco reveal structural chemical makeup in solanum xanthocarpum ultimately indicating therapeutic applications.

Vibration of the peptide bond containing C=O and N–H stretching , appearing between 1700 cm−1 and 1600 cm−1 provided information about protein, and vibration of C-O at 1200 cm−1 to 1300cm-1 is due to linkage with sugar molecule which is surrounded to Rubisco.

In order to compare spectral changes occurring in the rubisco, average spectra between control and treated with various acid shown in Table 3. The particular molecular interaction changes significantly in the compartment denoted in the form of shifting spectra from 1550 cm−1 –1600 cm−1 , 1350 cm−1 –1450 cm−1 , less than 1000 cm−1 , which is associated with a change in the construction. The active site of Rubisco are formed at the interfaces of adjutant subunit and catalytically functional amino acid residue reduced of lysine. The analogues of it was studied by Marl Herpel[23]. The interaction between analogues and lysine residue may alter the ribulose-1,5-bisphosphate carboxylase/oxygenase.

The Carbonyl region (1700–1750 cm^{-1}) and the other in the alkene absorption region (1630–1680 cm^{-1}). The latter was assigned to the enediol species formed as a result of enolization of acyclic *aldehydo* and *keto* forms of the sugars.

P=O stretch 1320-1140 cm-1 strong in IR, medium in Raman Substituents such as F or –OH cause the range to increase to 1415-1085 cm-1.

O=P-OH broad IR bands involving OH stretch from 2725-1600 cm-1, maxima at 2725 cm−1 -2525 cm-1 , 2350 cm−1 -2080 cm-1 , and 1740 cm−1 -1600 cm-1 ′ 1 –OH group, Peak at 1740 cm−1 -1600 cm-1 is strongest, P-O stretch at 1040 cm−1 -909 cm-1 ′ 2 –OH groups Peak at 1740-1600 cm-1 is weakest, if present at all, P-O stretches at 1030 cm−1 -972 cm-1 and 950-917 cm-1

P-CH_3 Asymmetrical deformation at 1450 cm−1 -1395 cm-1, Symmetric deformation at 1346 cm−1 -1255 cm-1, Rock at 977 cm−1 -842 cm-1 ′ P-CH_2-CH_3 and P-CH 2-R have deformation band at 1440 cm−1 -1400 cm-1 ′ P-Ar stretch 1130 cm−1 -1090 cm-1 medium in IR, weak in Raman ′ P-C stretch 754 cm−1 to 634 cm−1 medium weak in IR, strong in Raman, Interacts with other bonds on phosphorus; not very its characteristics.

Out-of-phase P-O-C stretch 1088 cm−1 to 920 cm-1 strong in IR, medium-weak in Raman (mostly C-O) , P-O-Et show second band ′ In-phase P-O-C stretch 845 cm−1 -725 cm-1 (mostly P-O) P=O stretch 1320 cm−1 -1140 cm-1 strong. Medium in Raman ′ O=P-OH broad IR bands involving OH stretch from 2725 cm−1 -1600 cm-1.

Strong IR stretch C=S1275 cm−1 -1030 cm-1, S=O 1225 cm−1 -980 cm-1, S-N ~700 cm-1 ′ C-S and S-H stretch stronger in Raman ′ S-S stretch not visible in IR or Raman ′ Strong P-H stretch 2440 cm−1 -2275 cm-1 ′ P=O stretch 1320-1140 cm-1 ′ Only see P-O stretch when R is small alkyl groups in P-O-R ′ O=P-OH broad IR bands involving OH stretch from 2725 cm−1 -1600 cm-1 ′ All P-O-C stretches and bends are below 1200 cm-1 ′ P-C deformation bands and stretches.

Thae all above data indicates that it change in its activity which paly vital role in chemotherapy of life.

4. CONCLUSION

Rubisco have specificity and potency which allow both detection and amplification of a target analyte to play very important role in photosynthesis. Rubisco exhibited catalytic efficiency and having multiple applications. After modification of Rubisco it opens avenues for protein engineering , biochemist, foodchemist, druggist and environmental scientist which will help to analyse for phytochemistry of C3and C4 plants.

5. REFERENCES

Xueliang Lyu, Cuicui Shen, Jiatao Xie, Yanping Fu, Daohong Jiang, Zijin Hu, Lihua Tang,Liguang Tang, Feng Ding, Kunfei Li, Song Wu, Yanping Hu, Lilian Luo, Yuanhao Li,Qihua Wang,Guoqing Li, and Jiasen Cheng , "A "footprint" of plant carbon fixation cycle functions during the development of a heterotrophic fungus", *Sci Rep*. 5: 12952, **(2015)**.

Lin MT, Occhialini A, Andralojc PJ, Parry MA·Hanson MR , "A faster Rubisco with

potential to increase photosynthesis in crops", *Nature* Sep 25; 513 (7519): 547-50, **(2014)**.

David Grossman, "A slow enzyme named rubisco has left plenty of room for improvem"**, (2019)**.

Goodsell, David S., "Rubisco: November 2000 Molecule of the Month." *RCSB Protein Data Bank*., **(2010).**

RuBisCo - Chemistry LibreTexts , https://chem.libretexts.org/Dec 30, **(2018)**.

Anna Vitlin Gruber and Leila Feiz, "Rubisco Assembly in the Chloroplast", Front. Mol. Biosci. **(2018).**

H. Aigner, R. H. Wilson, A. Bracher, L. Calisse, J. Y. Bhat, F. U. Hartl, M. Hayer-Hartl, "Plant RuBisCo assembly in *E. coli* with five chloroplast chaperones including BSD2", *Science* **(2017).**

So Young Lee, Puthanveettil Narayanankutty Damodaran and Kwang Soo Roh Saudi J Biol Sci., "Influence of salicylic acid on rubisco and rubisco activase in tobacco plant grown under sodium chloride *in vitro",*Nov; 21(5): 417–426, **(2014)**.

Orsolya Kinga Gondor, Rusina Yordanova, Gabriella Szalai, Magda Pál, "Salicylic acid and photosynthesis: signalling and effects", Acta Physiologiae Plantarum, Volume 36, Issue 10, pp 2537–2546, **(2014)**.

Roshy Joseph C, Ilanchezhian R , Patgiri BJ Ayurpharm**,** "Therapeutic Potentials of Kantakari (Solanum xanthocarpum Schrad. & Wendl.)" Int. J. Ayur Alli Sci., Vol.1, No.2 Pages 46- 53, **(2012)**.

O H Lowry, N J Rosebrough, A L Farr and R J Randall (1951), Protein measurement with the Folin-Phenol reagent. J.Biol.Chem. 193, 265-275

Wishnick M., Lane M.D.**,** "Ribulose disphosphate carboxylase from spinach leaves", Methods Enzymol. 23:570–577, **(1971)**.

Arnon DI., "Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris"*, *Plant Physiol*, vol.24 (pg. 1-15), **(1949).**

Bathellier C, Tcherkez G, Lorimer GH, Farquhar GD. Rubisco is not really so bad. Plant Cell Environ. **2018** Apr;41(4):705-716. doi: 10.1111/pce.13149. Epub 2018 Feb 28.

Edmondson DL, Badger MR, Andrews TJ (**1990)**, A kinetic characterization of slow inactivation of Ribulose bisphosphate carboxylase during catalysis. Plant Physiol 93, 1376-1382

Shimada M, Akamatsu Y, Tokimatsu T, Mii K, Hattori T., "Possible biochemical roles of oxalic acid as a low molecular weight compound involved in brown-rot and while-rot wood decays", *J. Biotechnol.*,vol. 53 pg. 103-113, **(1997)**.

Qing Wang Tongfei Lai Guozheng Qin Shiping Tian, "Response of Jujube Fruits to Exogenous Oxalic Acid Treatment Based on Proteomic Analysis", *Plant and Cell Physiology*, Volume 50, Issue 2, Pages 230–242, **(2009)**.

Juan David, "Guzman Natural Cinnamic Acids, Synthetic Derivatives and Hybrids with Antimicrobial Activity", Molecules 19, 19292-19349, **(2014)**.

Zhong Chen, Daniel R. Gallie, "Increasing Tolerance to Ozone by Elevating Foliar Ascorbic Acid Confers Greater Protection against Ozone Than Increasing Avoidance", Environmental Stress and Adaptation, Plant Physiology, Vol. 138, pp. 1673–1689, **(2005)**.

Tonsbeek in U.S. Patent. No. 3,615,600 **(1971).**

Lawrence L. Buckholz, Jr. Lewis G. Scharpf, "Aconitic acid, gluconic acid and/or succinic acid taken alone or further together with sclareolide in enhancing the organoleptic properties of foodstuffs", United States Patent 5366747, **(1994)**.

Kanjana Thumanua , Mathukorn Sompongb , Piyaporn Phansakc , Kanokwan Nontapotd and Natthiya Buensanteaib, "Use of infrared microspectroscopy to determine leaf biochemical composition of cassava in response to Bacillus subtilis", Journal of Plant Interactions, Vol. 10, No. 1, 270–279, **(2015)**.

Mark R. Harpel, and Fred C. Hartman, "Chemical Rescue by Exogenous Amines of a Site-Directed Mutant of Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase That Lacks a Key Lysyl Residue", *Biochemistry*, , *33* (18), pp 5553–5561, **(1994)**.