# Assessment of Anti-Oxidant Potency of Small Chain Glycopeptides Using DPPH Free Radical Scavenging Assay

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**Abstract:** 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) *in-vitro* assay was employed to determine the antioxidant potency of test compounds I to VII [Arg-Thr-Starch (RTStr); Ser-Arg-Lac (SRLac); Asn-Arg-Mannose (NRMs); Arg-Asn-Lac (RNLac); Arg-Thr-Lac (RTLac); His-Asn-Mannose (HNMs); Asn-His-Lac (NHLac)] using ascorbic acid as the standard drug. The percentage scavenging activity of the test drugs were determined at different concentrations and the $IC_{50}$ value of the test compounds were subsequently compared with that of ascorbic acid. Among the compounds tested, compound II (SRLac) showed highest antioxidant activity with an $IC_{50}$ value of 14.2 μg/ml whereas compounds I (RTS), VII (NHLac) and IV (RNLac) revealed the $IC_{50}$ value of 14.3 μg/ml, 14.5 μg/ml and 15.7 μg/ml, respectively when compared with ascorbic acid ($IC_{50}$ = 15.8 μg/ml). All the synthesized glycopeptides were further characterized by TLC, Melting point, IR, NMR and Mass spectral datas. Based on the above results, Ser-Arg-Lac could be considered as a lead compound for the development of new antioxidant drug for prevention of human diseases.

**Keywords:** 1.1-Diphenyl-2-picryl-hydrazyl, Antioxidant, Glycopeptides, Ascorbic acid, Potency.

## INTRODUCTION

Free radicals easily react with macro-molecules of crucial biological significance (DNA, lipids, protein) and destroy their structure and function, which accelerates ageing and might lead to degenerative diseases, including cancer [1, 2].

Certain portion of reactive oxygen species (ROS) is generated during normal human metabolism and the production rate is precisely controlled by specialized system of antioxidant defense [3]. This well-balanced ROS synthesis is impaired by inflammatory events, where activated macrophages and neutrophils, upon contact with pro-inflammatory stimuli, release substantial amounts of aggressive oxygen and nitrogen-centered radicals [4].

Natural antioxidant defense system involves enzymes (superoxide dismutase, Catalase, glutathione peroxidase), other proteins (albumin, ferritin, ceruloplasmin) and numerous smaller molecules (e.g. reduced glutathione, α-tocopherol, β-carotene, bilirubin, uric acid) having various modes of action. Antioxidants counteract ROS and diminish their deleterious effects [5, 6]. This protective barrier can be enhanced by the use of antioxidant micronutrient (vitamins C, E, β-carotene) and non-nutrient ingredients of edible plants, like polyphenols. Polyphenol subgroup of chemicals, flavonoids, is the extensively examined group of antioxidants [7, 8]. A new class of water soluble glycopeptide (PGY) fractionated and purified from the aqueous extracts of *Ganoderma lucidum* were evaluated with two conventional antioxidant testing systems of DPPH and superoxide radical scavenging and found to have their respective antioxidant activities in a concentration-dependent manner [9].

As part of discovery of natural antioxidant agents, a study was designed to investigate the in-vitro antioxidant activity of seven glycopeptides and in order to establish the most potent antioxidant drug having therapeutic values.

## MATERIALS & METHODS

### Chemicals

All chemicals were analytical grade. The chemicals required for biochemical assay were obtained from Sigma Chemicals Co., USA,

### DPPH Radical Scavenging Assay

The free radical scavenging activity of the test compounds was measured *in-vitro* by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay [10, 11]. About 0.3 mM solution of DPPH in 100% ethanol was prepared and 1ml of this solution was added to 3ml of test drug dissolved in ethanol at different concentrations (1-32

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ISSN: 2223-3806 / E-ISSN: 1927-5951/12 

**Table 1: DPPH Radical Scavenging Activiy of the Glycopeptide Leads**

| Test compound | % Inhibition | | | | | | *$IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|---|---|
| | 1µg/ml | 2µg/ml | 4µg/ml | 8µg/ml | 16µg/ml | 32µg/ml | |
| Compound I (RTStr) | 3.96 | 7.35 | 16.07 | 27.06 | 56.03 | 100 | 14.3 |
| Compound II (SRLac) | 4.83 | 11.59 | 19.14 | 30.60 | 55.63 | 100 | 14.2 |
| Compound III (NRMs) | 4.65 | 9.55 | 17.92 | 25.86 | 48.64 | 100 | 17.2 |
| Compound IV (RNLac) | 3.61 | 7.80 | 16.34 | 25.87 | 48.78 | 100 | 15.7 |
| Compound V (RTLac) | 3.24 | 6.70 | 12.34 | 21.64 | 46.71 | 100 | 17.1 |
| Compound VI (HNMs.) | 3.81 | 7.68 | 14.70 | 24.22 | 45.54 | 100 | 17.5 |
| Compound VII (NHLac) | 4.37 | 7.86 | 16.71 | 27.56 | 54.97 | 100 | 14.5 |
| Standard (Ascorbic Acid) | 4.47 | 8.96 | 14.83 | 25.86 | 50.29 | 100 | 15.8 |

*Values obtained from regression lines with 95% of confidence level. $IC_{50}$ is defined as the concentration sufficient to obtain 50% of a maximum effect estimate in 100%. All values given are mean of triplicate experiments at S.D. (5%) for the above table.

µg/ml). The mixture was shaken and allowed to stand at room temperature for 30 minutes and the absorbance was measured at 517 nm using UV-visible double beam spectrophotometer (Shimadzu-1800). The % scavenging activity at different concentrations of test drugs were determined and the $IC_{50}$ values were compared with that of ascorbic acid.

## Physico-Chemical Characterization

All the Synthesized glycopeptides were identified by their Melting point and TLC analysis as the preliminary level characterization and further confirmation of their structures was done with IR, $^{1}$H-NMR, $^{13}$C-NMR and Mass spectral analysis.

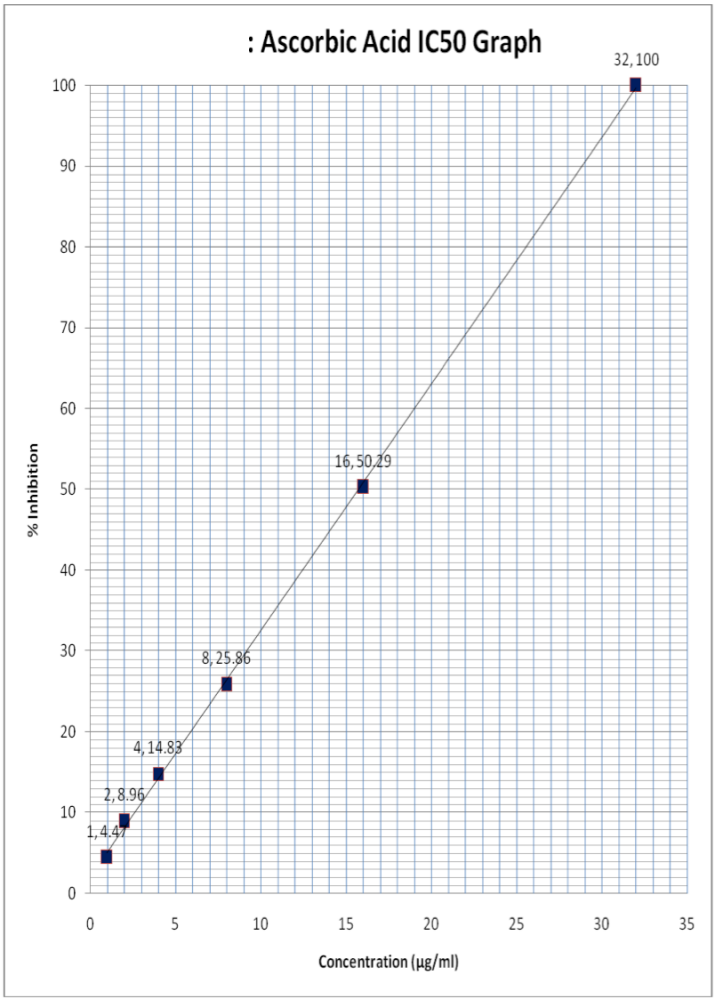


**Figure 1:** Graphical representation of Linearity for the Standard Drug Concentration with their Corresponding Value.

## RESULTS & DISCUSSION

### Antioxidant Capacity

The results of DPPH free radical scavenging activity of the test glycopeptides are shown in Table **1**.

The graphs of IC50 Value prediction in UV-visible spectrophotometric determination of the most potent test drug and the standard with their linear regression and correlation coefficient are shown in Figures **1**, **2** and **3**.

All the test drugs I-VII demonstrated H-donor activity. The highest DPPH radical scavenging activity was detected in test drug- II (Ser-Arg-Lac; SRLac) with an $IC_{50}$ value of 14.2 µg/ml (Table **1**), which showed that antioxidant activity was greater than that of standard ascorbic acid ($IC_{50}$ = 15.8 µg/ml).

Similarly test drugs- I (Arg-Thr-Starch; RTStr), VII (Asn-His-Lac; NHLac) and IV (Arg-Asn-Lac; RNLac) have shown comparatively better antioxidant capacity with that of the standard drug, ascorbic acid ($IC_{50}$ = 15.8 µg/ml) by their $IC_{50}$ values as 14.3 µg/ml, 14.5 µg/ml and 15.7µg/ml, respectively.

### Physico-Chemical Characterization

The Physico-chemical characterization of the synthesized glycopeptides are explained in Table **2-9**.

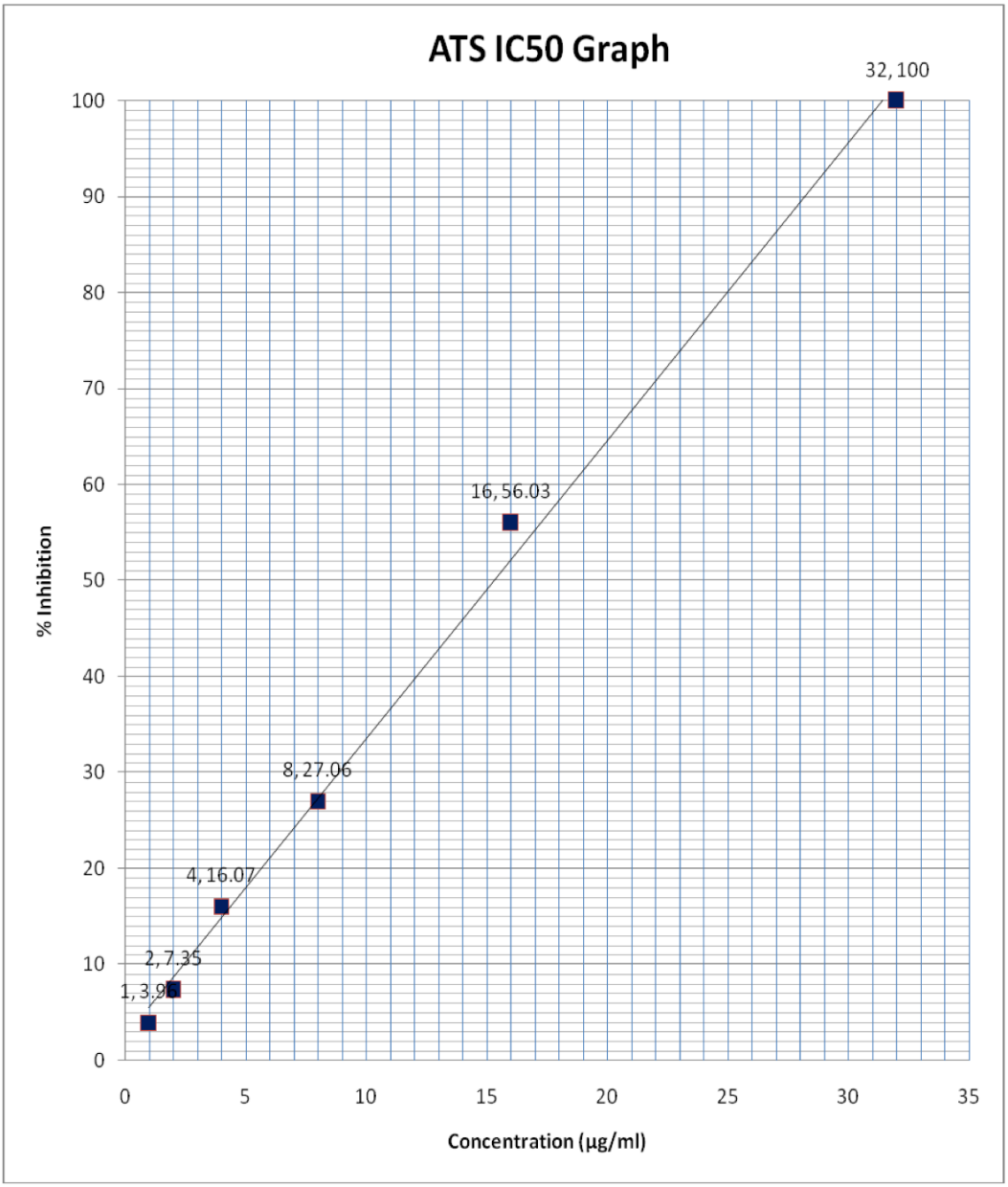


**Figure 2:** Graphical representation of Linearity for the Test Drug I (RTStr).

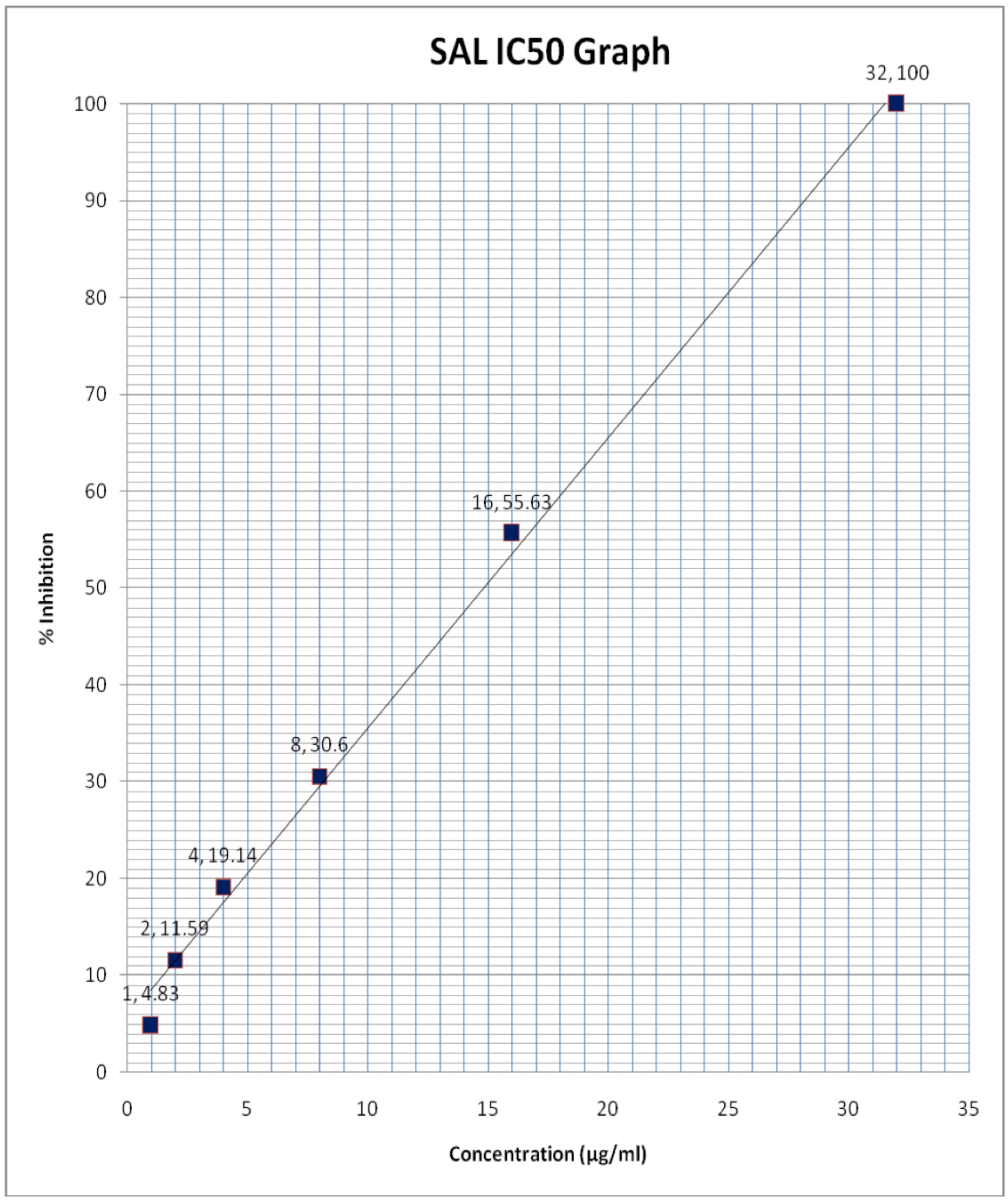


**Figure 3:** Graphical representation of Linearity for the Test Drug II (SRLac).

**Table 2: Preliminary Identification of the Synthesised Glycopeptidess by Melting Point & TLC Analysis**

| S. No. | COMPOUND NAME | M.Pt | Rf value (M.P.=$CHCl_3$:MeOH/9:1) |
|---|---|---|---|
| 1. | (SER-ARG-LAC) | 180°C | 0.80 |
| 2. | (ARG-THR-LAC) | 170°C | 0.30 |
| 3. | (HIS-ASP-MAN) | 180°C | 0.40 |
| 4. | (MAN-ARG-ASP) | 200°C | 0.45 |
| 5. | (LAC-HIS-ASP) | 160°C | 0.25 |
| 6. | (ARG-THR-STA) | 200°C | 0.60 |
| 7. | (ARG-ASP-LAC) | 190°C | 0.20 |

By this data, we conclude that compounds 2, 3, 6 & 7 gave sharp point on TLC and having 0.30, 0.40, 0.60 & 0.20 as $R_f$ values respectively with their above mentioned melting point values from the Table **2**.

**Table 3: Physico-Chemical Characterization of the O-Glycopeptide [Arg-Thr-Starch]**

| **I.R Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Wave number ($cm^{-1}$)** | **Characteristic functional gp. feasible** | **Compound type** |
| 1. | 1750 | C=0 st. | Carboxylic acids/amino acids |
| 2. | 1620 | N-H bend | Amines |
| 3. | 1570-1550 | N-H and N-C=0 (st.) sy. | Amides (amide IV) |
| 4. | 1100 | Cyclic ether st. asy. | Pyranose of sugar |
| 5. | 1000-940 | C-O-C st. sym. | Ether, acetal, ketal |
| 6. | 800 | Sym. st | Cyclic ether, acetal, ketal. |

| **$^{1}$H NMR Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Value (ppm)** | **Nature of peak** | **Significance and resemblance** |
| 1. | 8.082 | Doublet | R-C=O-NH- alk amide proton |
| 2. | 6.878 | Doublet | 1$^{st}$ 'OH' gp. Of glucose |
| 3. | 6.577 | Doublet | NH proton of $NH_2$-NH=NH |
| 4. | 4.402 | Small singlet | 'OH' gp. Of $CH_2$ side chain of glucose |
| 5. | 3.721 | Small singlet | Methine(CH) proton of OH |
| 6. | 3.410 | Small singlet | $CH_2$-O-$CH_2$ ether (α 1-4) link of glucose and alk-O- |
| 7. | 2.932 | Sharp singlet | Methylene($CH_2$) proton of OH |
| 8. | 1.20 | Singlet narrow | $CH_2$ proton |
| 9. | 0.85 | singlet | $CH_3$ proton |

| **$^{13}$C NMR Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Value (ppm)** | **Nature of peak** | **Significance and resemblance** |
| 1. | 110.791 | Singlet(w) | (O-C-O) of acetal/ketal in sugar |
| 2. | 39.757 | Singlet(s) | $CH_2$-C =O(groups of 2 or more equivalent carbons) |
| 3. | 31.195 | Singlet(m) | Alkyl amine carbon(C-N) |

| **MASS Spectral characterization:** | | | | | |
|---|---|---|---|---|---|
| **S. No.** | **Name of the ion** | **mass** | **Product ion and composition of the neutral particle lost** | **Substructure or compound type** | **Peak m/z ratio (mol.wt:899)** |
| 1. | $H_2O$+. | 18 | [M-18]+ ($H_2O$) | Non specific, abundant: alcohols, some acids, aldehydes, ketones, lactones, cyclic ether 'O'indicator | 881 |

**Table 4: Physico-Chemical Characterization of the N-Glycopeptide [Arg-Asn-Lactose]**

| **I.R Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Wave number ($cm^{-1}$)** | **Characteristic functional gp. feasible** | **Compound type** |
| 1. | 1660-1625 | N-H s bend<br>C=O st.<br>C=O st. (amides I) | Amines,<br>Aldehyde, acids<br>Amides |
| 2. | 1660-1510 | N-H s and N-C=0 (st.) sy. (Amide II) | Amides |
| 3. | 1575-1510 | $N^{+}$-H s bend | Amines |
| 4. | 1250-1100 | C-O-C st. Cyclic ether st. asy. | Ethers |
| 5. | 1100 | C-O-(H)st. | Alcohols<br>Ethers |

**(Table 4). Continued.**

| **$^{1}$H NMR Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Value (ppm)** | **Nature of peak** | **Significance and resemblance** |
| 1. | 8.082 | Doublet, broad | R-C=O-NH- alk amide proton |
| 2. | 6.997 | Doublet, broad | 1$^{st}$ 'OH' gp of glucose |
| 3. | 6.584 | Doublet, narrow | NH proton of $NH_2$-NH=NH |
| 4. | 5.029 | Small singlet | 3$^{rd}$ 'OH' gp of lactose (glucose) |
| 5. | 4.401 | singlet | 'OH' gp of $CH_2$ side chain of lactose |
| 6. | 3.721 | Sharp singlet | Methine(CH) proton of OH |
| 7. | 3.235 | Singlet narrow | $CH_2$-O-$CH_2$ ether (α 1-4) link of glucose |
| 8. | 3.163 | Sharp singlet | Methylene($CH_2$) proton of OH |
| 9. | 2.494 | Sharp singlet | $NH_3^+$ proton (alkyl/amine) |
| 10. | 1.5 | Small singlet | CH proton |
| 11. | 1.2 | singlet | $CH_2$ proton |

| **$^{13}$C NMR Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Value (ppm)** | **Nature of peak** | **Significance and resemblance** |
| 1. | 207.067 | Small singlet (w) | Aldehyde of sugar & ketonic carbon |
| 2. | 175.694 | Singlet (m) | Amides |
| 3. | 171 | Singlet (w) | Carboxylate anion |
| 4. | 162.802 | Singlet (w) | -C=O of H-C=O-NH- |
| 5. | 118.112 | Singlet (s) | (O-C-O) of acetals/ketals |
| 6. | 79.739 | Singlet (w) | 'OH' bearing carbon of sugar |
| 7. | 62.935 | Singlet (m) | Etheral carbon of connecting sugars |
| 8. | 55.460 | Singlet (m) | $CH_2$ of OH in sugars |
| 9. | 39.651 | Septet (s) | $CH_2$ –C=O-(Groups of 2 or more equivalent carbons) |
| 10. | 31.182 | Singlet (m) | Alkyl amines(C-N) |
| 11. | 25.652 | Singlet (m) | NH of CONH |
| 12. | 24.922 | Singlet (w) | Cyclic ether |

W= weak signal intensity, M= moderate signal intensity.

| **MASS Spectral characterization:** | | | | | |
|---|---|---|---|---|---|
| **S. No.** | **Ion name** | **mass** | **Product ion and composition of the neutral particle lost** | **Substructure or compound type** | **Peak m/z ratio (mol.wt:629)** |
| 1. | $C_2H^+$ | 25 | $[M-25]^{+\cdot}$ ($C_2H$) | Terminal acetylenyl | 604 |

**Table 5: Physico-Chemical Characterization of the N-Glycopeptide [Mannose-Arg-Asn]**

| **I.R Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Wave number (cm$^{-1}$)** | **Characteristic functional gp. feasible** | **Compound type** |
| 1. | 1740 | C=O st. | Amino acids |
| 2. | 1740-1650 | C=O st. | Carboxylic acids |
| 3. | 1670-1650 | C=O st. (Amide I) | Amides |
| 4. | 1650-1550 | N-H s bend C-O-C st. Cyclic ether st. asy. | Amines |
| 5. | 1590 | N-H s & N-C=O st. sy. (Amide II) | Amides |
| 6. | 1590-1510 | $N^+$-H s bend | Amines |
| 7. | 1450 | $CH_2$ –(C=C) s bend<br>$CH_2$ s bend | Alkenes<br>Alkanes |
| 8. | 1100 | C-O (H) st. | Alcohols |
| 9. | 760-500 | $CH_2$ bend | Alkanes |

**(Table 5). Continued.**

| **$^{1}$H NMR Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Value (ppm)** | **Nature of peak** | **Significance and resemblance** |
| 1. | 8.115 | Singlet, broad | R-C=O-NH- alk amide proton |
| 2. | 6.652 | Doublet | NH proton of $NH_2$-NH=NH |
| 3. | 4.400 | Singlet | Amid methine proton |
| 4. | 3.721 | Sharp singlet | Methine(CH) proton of OH |
| 5. | 3.311 | singlet | Methylene($CH_2$) proton of OH |
| 6. | 2.978 | Sharp singlet | $NH_2$ proton |
| 7. | 2.495 | Sharp singlet | $NH_3^+$ proton (alkyl/amine) |
| 8. | 1.9 | Singlet narrow | Methine(CH) proton attached with =N in sugar |
| 9. | 1.229 | Singlet narrow | Methylene($CH_2$) proton |

| **$^{13}$C NMR Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Value(ppm)** | **Nature of peak** | **Significance and resemblance** |
| 1. | 113.865 | Singlet (m) | (O-C-O) of acetal/ketal in sugar |
| 2. | 55.473 | Singlet (w) | $CH_2$ of OH in sugar |
| 3. | 39.914 | Singlet (s) | $CH_2$-C=O- (groups of 2 or more equivalent carbons) |
| 4. | 31.182 | Singlet (m) | Alkylamines (C-N) |

W= weak signal intensity, M= moderate signal intensity, S= strong signal intensity.

| **MASS Spectral characterization:** | | | | | |
|---|---|---|---|---|---|
| **S. No.** | **Name of the ion** | **mass** | **Product ion and composition of the neutral particle lost** | **Substructure or compound type** | **Peak m/z ratio (mol.wt:451)** |
| 1. | $CH_3^+$ | 15 | $[M-15]^{+\cdot}$ ($CH_3$) | Non specific, abundant: Methyl, N-ethylamine | 436 |

**Table 6: Physico-Chemical Characterization of the N-Glycopeptide [His-Asn-Mannose]**

| **I.R Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Wave number ($cm^{-1}$)** | **Characteristic functional gp. feasible** | **Compound type** |
| 1. | 1675-1650 | C=O st.<br>C=O st.<br>C=O st. | Amides (amide I)<br>Acids<br>Alkene & cyclo alkene |
| 2. | 1650-1525 | ar. C-C st. | Hetero aromatic compounds |
| 3. | 1650-1550 | N-H s bend | Amines |
| 4. | 1610-1550 | N-H s bend N-C=O st. sy. | Amides |
| 5. | 1525 | $N^+$-H s bend | Amines |
| 6. | 1000 | C-O(H) st. | Alcohols |
| 7. | 1000-800 | H –(C=C) s oop. Bend | Alkene, cycloalkene |
| 8. | 750-500 | $CH_2$ s bend | Alkanes |

**(Table 6). Continued.**

| **$^{1}$H NMR Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Value (ppm)** | **Nature of peak** | **Significance and resemblance** |
| 1. | 8.100 | Singlet, broad | R-C=O-NH- alk amide proton |
| 2. | 7.226 | Doublet | Proton between N & NH in ring imidazole |
| 3. | 6.877 | Doublet | $NH_3^+$ proton |
| 4. | 6.604 | Doublet | Imidazole ring proton adjacent to N |
| 5. | 5.0 | Small singlet | Amide methane proton adjacent to $CH_2$ side chain of imidazole |
| 6. | 4.401 | Sharp singlet | Methylene proton adjacent to imidazole ring |
| 7. | 3.9 | Small singlet | Methylene proton adjacent to NH of imidazole ring |
| 8. | 3.720 | Sharp Singlet | methine proton of 'OH' |
| 9. | 2.948 | Sharp Singlet | Methylene proton of 'OH' |
| 10. | 1.9 | Small singlet | Methane (CH) proton attached with =N in sugar |
| 11. | 1.2 | Small singlet, narrow | $CH_2$ proton |

| **$^{13}$C NMR Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Value (ppm)** | **Nature of peak** | **Significance and resemblance** |
| 1. | 128.37 | Singlet (m) | Carbon(a,b) in heteroaromatic imidazole ring system |
| 2. | 113.88 | Singlet (m) | (O-C-O) of acetal/ketal in sugar |
| 3. | 55.46 | Singlet (w) | $CH_2$ of OH in sugar |
| 4. | 39.65 | Singlet (s) | $CH_2$-C=O- (groups of 2 or more equivalent carbons) |
| 5. | 25.31 | Singlet (m) | Amine carbon |

W= weak signal intensity, M= moderate signal intensity, S= strong signal intensity.

| **MASS Spectral characterization:** | | | | | |
|---|---|---|---|---|---|
| **S. No.** | **Name of the ion** | **mass** | **Product ion and composition of the neutral particle lost** | **Substructure or compound type** | **Peak m/z ratio (mol.wt:432)** |
| 1. | $C_4H_8O^{+\cdot}$ | 72 | $[M-72]^{+\cdot}$ alkanes | 'O' indicator | 360 |

**Table 7: Physico-Chemical Characterization of the N-Glycopeptide [lactose-His-Asn]**

| **I.R Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Waveno. ($cm^{-1}$)** | **Characteristic functional gp. feasible** | **Compound type** |
| 1. | 1750 | C=O st. & C=O st. | Carboxylic acids & Carboxylic ester |
| 2. | 1650 | C=O st.<br>C=O st. | Amide(amide I)<br>Alkenes & cycloalkenes |
| 3. | 1620 | N-H s bend | Amines |
| 4. | 1590 | N-H s bend N-C=O st. sy. | Amides (amide II) |
| 5. | 1510 | $N^+$-H s bend | Amines |
| 6. | 1450 | Ar. C-C st.<br>$CH_2$ –(C=C) s bend<br>$CH_2$ s bend | Heteroaromatic compounds<br>Alkenes & cycloalkenes<br>alkanes |
| 7. | 1250 | C-O-C st. asy. | Ethers |
| 8. | 1110 | C-O(H) st. | Alcohols |
| 9. | 960-700 | Ar. C-H s oop. Bend<br>H –(C=C) s oop. Bend | Heteroaromatic compounds<br>Alkenes & cycloalkenes |
| 10. | 680 | $CH_2$ s bend | Alkanes |

**(Table 7). Continued.**

| **$^{1}$H NMR Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Value (ppm)** | **Nature of peak** | **Significance and resemblance** |
| 1. | 8.116 | Singlet, broad | R-C=O-NH- alk amide proton |
| 2. | 7.204 | Doublet | Imidazole ring proton |
| 3. | 7.018 | Doublet, small | 1$^{st}$ 'OH' gp of glucose |
| 4. | 6.604 | Doublet | $NH_3^+$ proton |
| 5. | 6.652 | Doublet | Imidazole ring proton |
| 6. | 5.020 | Small singlet | 3$^{rd}$ 'OH' gp of lactose-glucose |
| 7. | 4.399 | Sharp singlet | 'OH' gp. of $CH_2$ side chain of lactose & Amide CH proton (a) |
| 8. | 3.894 | Small Singlet | methylene proton of (b) |
| 9. | 3.721 | Sharp Singlet | Methine proton of 'OH' |
| 10. | 3.315 | Broad singlet | $CH_2$ –O- $CH_2$ ether linkage proton of lactose |
| 11. | 2.978 | Sharp singlet | methylene proton of (OH) |
| 12. | 1.7 | Small Singlet | CH proton |
| 13. | 1.227 | singlet, narrow | $CH_2$ proton |

| **$^{13}$C NMR Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Value (ppm)** | **Nature of peak** | **Significance and resemblance** |
| 1. | 207 | Singlet (w) | Aldehyde of sugar & ketonic carbon |
| 2. | 128.366 | Singlet (m) | Carbon (a,b) in heteroaromatic imidazole ring system |
| 3. | 113.867 | Singlet (m) | (O-C-O) of acetal/ketal in sugar |
| 4. | 62.933 | Singlet (w) | Ethereal carbon of connecting sugars |
| 5. | 55.461 | Singlet (w) | $CH_2$ of OH in sugar |
| 6. | 39.924 | Singlet (s) | $CH_2$-C=O- (groups of 2 or more equivalent carbons) |
| 7. | 31.176 | Singlet (m) | Amine carbon |
| 8. | 23.536 | Singlet (w) | Cyclic ether |

W= weak signal intensity, M= moderate signal intensity, S= strong signal intensity.

| **MASS Spectral characterization:** | | | | | |
|---|---|---|---|---|---|
| **S. No.** | **Name of the ion** | **mass** | **Product ion and composition of the neutral particle lost** | **Substructure or compound type** | **Peak m/z ratio (mol.wt:602)** |
| 1. | $C_3H_7O_2^{+\cdot}$ | 75 | $[M-75]^{+\cdot}$ | Methyl acetyl<br>2 X 'O' indicator | 527 |

**Table 8: Physico-Chemical Characterization of the O-Glycopeptide [Arg-Thr-Lac]**

| **I.R Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Wave number (cm$^{-1}$)** | **Characteristic functional gp. feasible** | **Compound type** |
| 1. | 1670 | C=O st. | Aldehyde |
| 2. | 1650 | C=O st. | Amide(amide I) & lactams |
| 3. | 1625 | N-H s bend<br>N-H s bend N-C=O st. sy. | Amines<br>Amides (amide II) |
| 4. | 1400 | $CH_3$ s sy. | alkanes |
| 5. | 1160 | C-O (H) st.<br>C-O-C st. asy. | Alcohols<br>Ethers |
| 6. | 750 | $CH_2$ s | alkanes |

**(Table 8). Continued.**

| **$^{1}$H NMR Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Value (ppm)** | **Nature of peak** | **Significance and resemblance** |
| 1. | 8.124 | Singlet, narrow | R-C=O-NH- alk amide proton |
| 2. | 6.876 | Doublet | 1$^{st}$ 'OH' gp of glucose |
| 3. | 6.713 | Singlet, narrow | NH proton of $NH_2$-NH=NH |
| 4. | 4.405 | Singlet, narrow | 'OH' gp. of $CH_2$ side chain of glucose & galactose |
| 5. | 3.723 | Small singlet | Methine proton of 'OH' |
| 6. | 3.307 | Sharp singlet | $CH_2$ –O- $CH_2$ ether linkage proton of disaccharide |
| 7. | 2.495 | Sharp singlet | $NH_3^+$ proton |
| 8. | 1.229 | Singlet | $CH_2$ proton |
| 9. | 0.8 | Small Singlet | $CH_3$ proton |
| 10. | -0.072 | Singlet, narrow | Proton of integral reference standard TMS |

| **$^{13}$C NMR Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Value (ppm)** | **Nature of peak** | **Significance and resemblance** |
| 1. | 207.087 | Singlet (w) | Aldehyde of sugar & ketonic carbon |
| 2. | 113.862 | Singlet (w) | (O-C-O) of acetal/ketal in sugar |
| 3. | 39.922 | Singlet (s) | $CH_2$ –C=O- (groups of 2 or more equivalent carbons) |
| 4. | 31.198 | Singlet (m) | Alkyl amines(C-N) |

W= weak signal intensity, M= moderate signal intensity, S= strong signal intensity.

| **MASS Spectral characterization:** | | | | | |
|---|---|---|---|---|---|
| **S. No.** | **Name of the ion** | **mass** | **Product ion and composition of the neutral particle lost** | **Substructure or compound type** | **Peak m/z ratio (mol.wt:628)** |
| 1. | $K^+$ | 39 | $[M-39]^{+\cdot}$ Molecular ion with $K^+$ adduct | Sometimes strong even if $K^+$ is only an impurity | 667 |

**Table 9: Physico-Chemical Characterization of the O-Glycopeptide [Ser-Arg-lactose]**

| **I.R Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Wave number ($cm^{-1}$)** | **Characteristic functional gp. feasible** | **Compound type** |
| 1. | 1740 | C=O st.<br>C=O st.<br>C=O st. | Aldehydes<br>Esters<br>ketones |
| 2. | 1670 | C=O st. (amide I) | Carboxylic amide & lactam |
| 3. | 1625 | N-H s bend | Amines |
| 4. | 1600 | N-H s bend N-C=O st. sy. (amide II) | Amides & lactams |
| 5. | 1470 | $N^+$-H s bend<br>$CH_2$ s bend | Amines<br>alkanes |
| 6. | 1260 | C-O-C st. asy. | Ethers |
| 7. | 1000 | C-O (H) st. | Alcohols |

**(Table 9). Continued.**

| **$^{1}$H NMR Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Value (ppm)** | **Nature of peak** | **Significance and resemblance** |
| 1. | 7.821 | Doublet | R-C=O-NH- alk amide proton |
| 2. | 6.925 | Singlet, broad | 1$^{st}$ 'OH' gp of glucose |
| 3. | 4.8 | Small singlet, broad | 3$^{rd}$ 'OH' gp of lactose-glucose |
| 4. | 4.4 | Small singlet | 'OH' gp. of $CH_2$ side chain of lactose |
| 5. | 4.006 | triplet | Amide methine proton of 'OH' |
| 6. | 3.406 | Singlet narrow | $CH_2$–O- $CH_2$ ether linkage proton of lactose |
| 7. | 3.160 | Sharp singlet | Methylene proton of 'OH' |
| 8. | 2.494 | Sharp singlet | $NH_3^+$ proton (alkyl amines ) |
| 9. | 1.6 | Small Singlet, broad | CH proton |
| 10. | 1.103 | triplet | $CH_2$ proton |
| 11. | 0 | Small, singlet | Proton of integral reference standard TMS |

| **$^{13}$C NMR Spectral characterization:** | | | |
|---|---|---|---|
| **S. No.** | **Value (ppm)** | **Nature of peak** | **Significance and resemblance** |
| 1. | 114.029 | Singlet (w) | (O-C-O) of acetal/ketal in sugar |
| 2. | 39.913 | Septet (s) | $CH_2$–C=O- (groups of 2 or more equivalent carbons) |
| 3. | 31.190 | Singlet (m) | Alkylamines(C-N) |

W= weak signal intensity, M= moderate signal intensity, S= strong signal intensity.

| **MASS Spectral characterization:** | | | | | |
|---|---|---|---|---|---|
| **S. No.** | **Name of the ion** | **mass** | **Product ion and composition of the neutral particle lost** | **Substructure or compound type** | **Peak m/z ratio (mol.wt:614)** |
| 1. | $C_5H_6^{+\cdot}$ | 66 | $[M-66]^{+\cdot}$ ($C_5H_6$) | cyclopentenes | 548 |

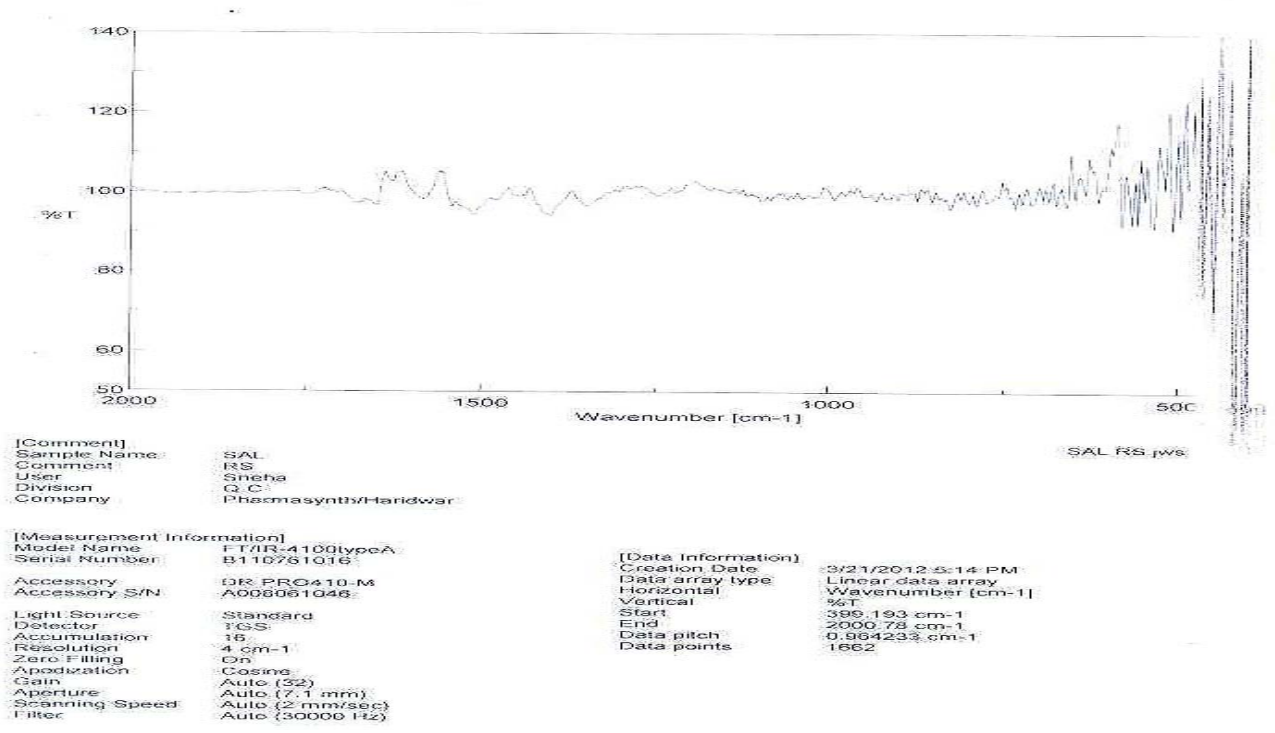


**Figure 4:** I.R spectra of Ser-Arg-Lac.

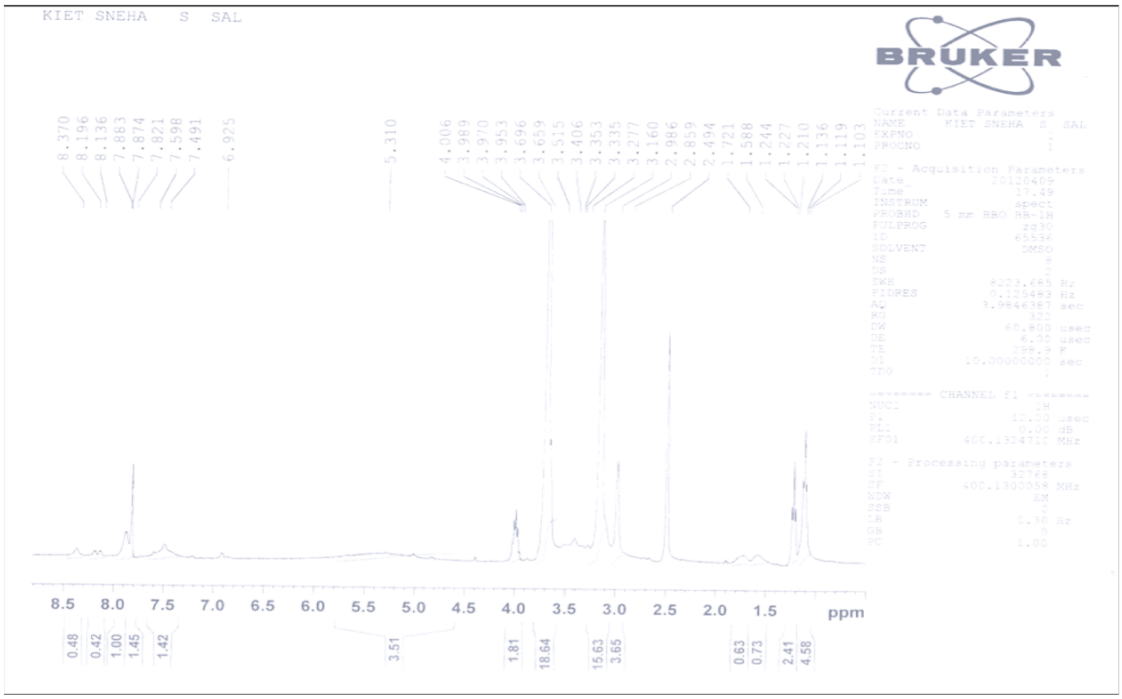


**Figure 5:** [1]H-NMR spectra of Ser-Arg-Lac.

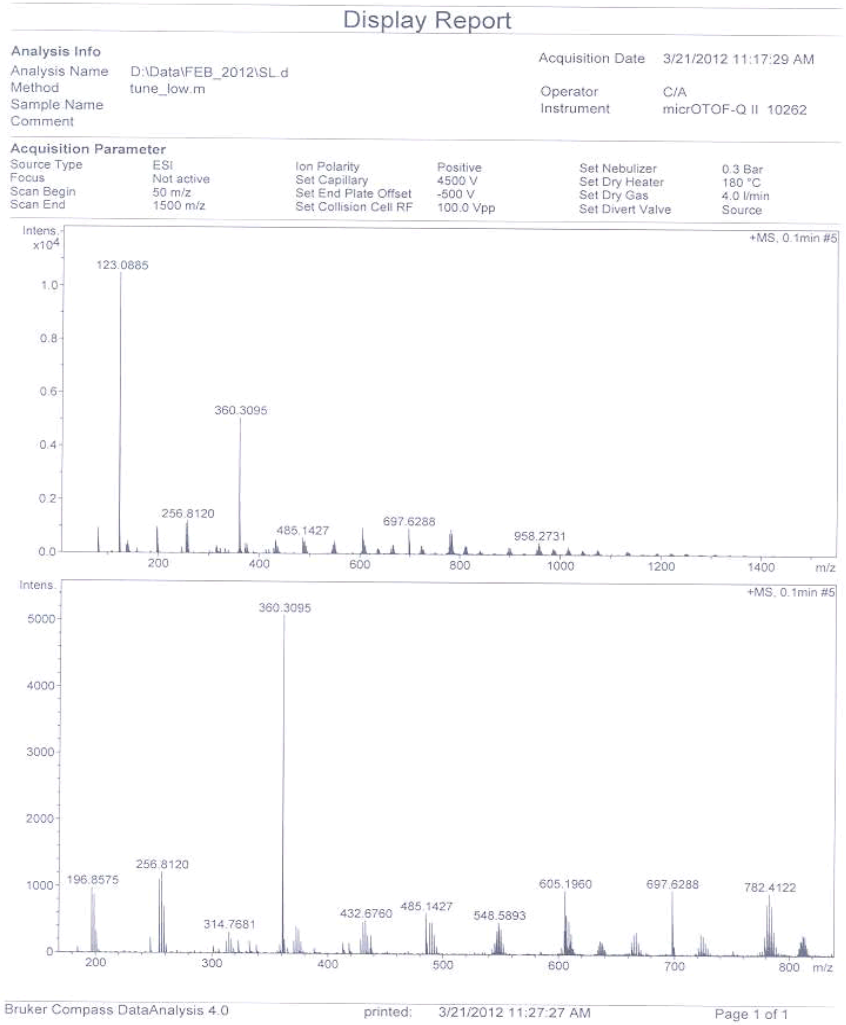


**Figure 6:** Mass Spectra of Ser-Arg-Lac.

All the spectral characterization of the synthesized glycopeptides was expressed in Table **2-9** above and with their significance and the compound type after fragmentation. The I.R, $^{1}$H-NMR, and Mass spectral scan of the most potent test compound Ser-Arg-Lac (SRLac) are shown in Figures **4** to **6**.

Antioxidants are the compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, ageing, atherosclerosis, ischemic injury and inflammation and neuro degenerative diseases. The above studies suggest that test drugs II, I and VII (SRLac, RTStr and NHLac) possess remarkable antioxidant property that may maintain good health by boosting the immune system and reducing inflammation and allergic reactions.

It can be concluded that test drug II (Ser-Arg-Lac; SRLac) could be considered as a possible candidate for further studies for nutraceutical preparations with potent antioxidant activities for the prevention of human diseases.

## ACKNOWLEDGEMENT

The authors are very much thankful to Dr. S. Narendra Kumar, Director, KIET Group of Institutions, Ghaziabad, India. Also, we remain thankful to Mrs. M. Uma Maheswari, Professor, Department of Pharmacology, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, India for her valuable technical suggestions.

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Received on 27-08-2012 Accepted on 20-09-2012 Published on 05-11-2012

DOI: http://dx.doi.org/10.6000/1927-5951.2012.02.02.10