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Research paper

In vitro antioxidant potency of some smaller chain glycopeptides with the prediction of IC50 values

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Abstract

Hydrogen peroxide, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) and Phosphomolybdenum in-vitro assay was employed to determine the antioxidant potency of glycopeptides RN Mannose, RK starch, RNRN Mannose and RHRCR Starch using ascorbic acid as the standard drug. The percentage scavenging activity of the glycopeptides were determined at different concentrations and the IC50 value of the test compounds were subsequently compared with that of ascorbic acid. RN Mannose was found to be most potent antioxidant compound. Also, Swiss dock study was performed with three glycopeptides, viz., RHRCR Mannose, RN Mannose and RNRN Mannose.Among these, RHRCR Mannose was found to have the best affinity for the receptor with stearic energy -0.2306kcal/mol.

Keywords: *Glycopeptides; Docking; Antioxidant Assay; ROS; Oxidative Stress.*

1. Introduction

Antioxidants are the compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. They donate their own electrons to free radicals thereby inhibiting the chain reactions that can cause cell damage. When the generation of these species exceed the levels of antioxidants mechanism, they cause extensive damage to cells leading to oxidative damage of tissue and biomolecules, eventually leading to disease conditions, especially leading to disease conditions like cancer, cardiovascular disease, neural disorder, Alzheimer's disease, aging, etc (Thomas and Kalyanaraman, 1997; Beckman and Ames, 1998).

Oxidative stress is an imbalanced state where excessive quantities of reactive oxygen and/or nitrogen species (ROS/RNS, e.g., superoxide anion, hydrogen peroxide, hydroxyl radical, and peroxynitrite) overcome endogenous antioxidant capacity, leading to oxidation of varieties of bio-macromolecules, such as enzymes, proteins, DNA and lipids (Valko et al., 2004). Oxidative stress is important in the development of chronic degenerative diseases including coronary heart disease, cancer and aging (Halliwell, 1997).

Reactive oxygen species (ROS) are produced as a consequence of normal aerobic metabolism or host defence mechanism. Excessive free radical species attack cellular components that cause damage to lipids, proteins, and DNA, which may initiate a chain of events resulting in the onset of a variety of diseases (Flora, 2007).

Recently, among the many antioxidants of various origins used in food industry (Andrea et al. 2010), peptide antioxidants attract great interest (Davalos et al., 2004; Chen et al., 1995; Hernandez-Ledesma et al., 2005 and Pihlanto, 2006). The aim of this work is a comparative study of the antioxidant activity of some smaller chain glycopeptides.

2. Materials and methods

2.1. Chemicals

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

2.2. Swiss dock software and target protein for docking

Swiss Dock was used for docking of selected glycopeptides viz., RHRCR Mannose, RN Mannose and RNRN Mannose with Peroxiredoxi-5, mitochondrial, PRDX5. It is a protein that is encoded by gene, PRDX5 which play an antioxidant role in different tissues and during inflammatory condition. These reduce the H_2O_2 and alkyl hydro peroxides by interacting with peroxisome receptor1. Docking studies helps in prediction of the preferred orientation of

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a ligand with the binding site on a protein. Molecular docking was used to determine appropriate binding orientations and conformations of various chemical compounds at the target site. After docking, all the legend confirmations were ranked on the basis of their binding energy.

2.3. Antioxidant assays

2.3.1. Hydrogen peroxide scavenging method

The hydrogen peroxide scavenging assay was carried out following the procedure of Jayaprakasha et al. (2004). This method is based on the reduction of H_2O_2,a weak oxidising agent which can serve as a possible source of •OH free radical. A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Different dilutions of test compound in distilled water (1, 2, 4, 8, 16,32 µg/ml) were added to a hydrogen peroxide solution (1.0 mL, 40mM) and after 10mn the absorbance of the reaction mixture was recorded at 230 nm against a blank solution containing the phosphate buffer without hydrogen peroxide (Miller et al., 1993; Jayaprakasha et al., 2004).
The percentage of hydrogen peroxide scavenging of both test compounds and standard compounds were calculated:

$$\% \text{ Scavenging } [H_2O_2] = [(A_C - A_S)/A_C] \times 100$$

Where Ac was the Absorbance of control reaction
As was the Absorbance of test or standard sample.
The IC_{50} value of the samples, was calculated using dose inhibition curve.Lower absorbance of the reaction mixture indicated higher free radical activity.

2.3.2. DPPH Free radical scavenging assay

The free radical scavenging activity of test compounds and ascorbic acid as positive control was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. 0.1 mM solution of DPPH in ethanol was prepared. 1 ml of this solution was added to 3 ml. of different concentration of test compound (1, 2,4,8,16,32 µg/ml).The mixture was shaken vigorously and allowed to stand at room temp for 30 min and absorbance was measured at 517 nm by using a spectrophotometer (Shimadzu 1800 UV-visible spectrophotometer). Similar procedure was followed with ascorbic acid.
The degree of decolorization of DPPH from purple to yellow indicated the scavenging efficiency of the test compounds and ascorbic acid. (Blios, 1958; Warrier et al., 1994).
The percentage inhibition of DPPH free radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging effect (\%) or Percent inhibition} = A_c - A_s / A_c \times 100.$$

Where A_c was the Absorbance of control reaction
A_s= Absorbance of test or standard
The % inhibition data was then plotted against log concentration fitted in a graph and IC_{50} (half-maximal inhibitory concentration) value was calculated by linear regression analysis
Lower absorbance of the reaction mixture indicated higher free radical activity.

2.3.3. Phosphomolydenum method

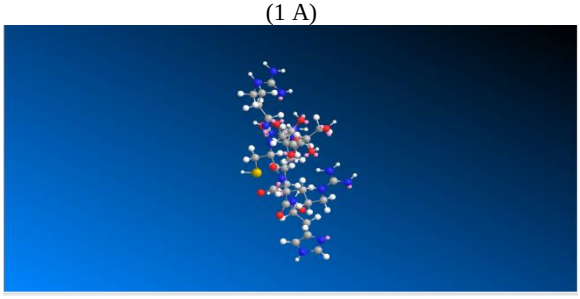
The Phosphomolybdenum, PM assay, used for determining the antioxidant capacity is based on the reduction of Mo (VI)–Mo (V) by the antioxidants and subsequent formation of a green phosphate/Mo (V) complex at acid pH, that shows maximum absorption at 695nm. It gives a direct estimation of reducing capacity of antioxidant. The method was conducted according to the procedure described by Prieto et al., 1999. 0.3ml of test sampleof different concentrations were taken in a tube and mixed with 3 ml of reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate and incubated in water bath at 95°C for 90 min. After cooling to room temperature, the absorbance of the solutions were measured at 695nm using a spectrophotometer with water as a blank. Ascorbic acid was utilized as a reference standard. The antioxidant activity was expressed as the number of gram equivalents of ascorbic acid.

3. Results and discussion

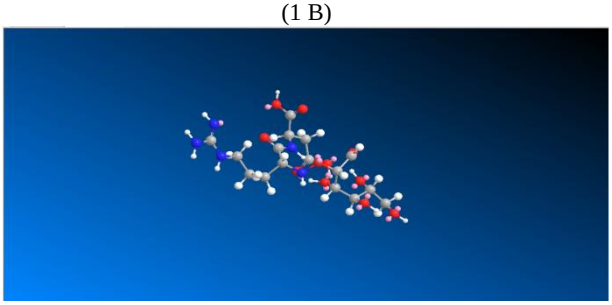
3.1. Swiss dock study

Binding energy for each docking was calculated using a semi-empirical free energy force field. Out of these 3 docked molecules with the receptor (code 4MMM), RHRCR Mannose was found to have the best affinity for the receptor with stearic energy (-0.2306kcal/mol). The results are shown in fig. 1 and stearic enegy of the glycopeptides are given in table 1.

(1 A)



(1 B)



(1 C)

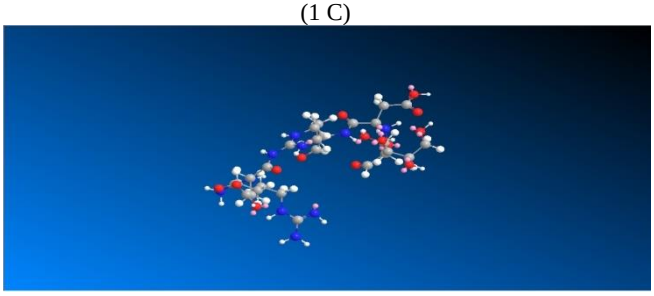


Fig. 1: Swiss Docking of Glycopeptides (1a: RHRCR Mannose; 1b: RN Mannose; 1c: RNRN Mannose)

Table 1: Stearic Energies of Glycopeptides Calculated from Swiss Dock

Compound	Stearic energy
RHRCR Mannose	-0.2306kcal/mol
RN Mannose	-12.457kcal/mol.
RNRN Mannose	-7.226kcal/mol.

3.2. Antioxidant assays

In this study, the antioxidant activity of different glycopeptides were compared with ascorbic acid. Various in vitro tests i.e. H_2O_2 radicals scavenging, DPPH free radical scavenging activity, andtotal antioxidant activity (Phosphomolybdenum method) were conducted for investigating its antioxidant potency.

3.2.1 Hydrogen peroxide scavenging method

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly and inside the cell probably reacts with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radical which may be the origin of many of its toxic effects (Miller et al., 1993). It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate.

The scavenging ability of some smaller chain glycopeptides and ascorbic acid was compared and shown in Table2 concentration (µg/ml) and Fig.2. The scavenging activity of the glycopeptides were observed in a concentration dependant manner and was comparable to that of the standard, ascorbic acid. IC_{50} values are reported in Table 3.

Table 2: Percent Inhibition of Glycopeptides and Ascorbic Acid by Hydrogen Peroxide Scavenging Assay.

Compound	1µg/ml	2 µg/ml 2	4 µg/ml	8 µg/ml	16 µg/ml	3232 µg/ml
RN Mannose	28.39	32.09	37.03	39.5	39.50	46.91
RK Starch	16.04	19.75	18.51	22.2	24.69	30.86
RNRN Mannose	46.91	49.38	51.85	53.08	55.55	56.79
RNRCR Mannose	8.6	11.11	11.11	13.58	16.04	18.51
Ascorbic acid	33.33	35.80	38.27	41.9	45.67	49.38

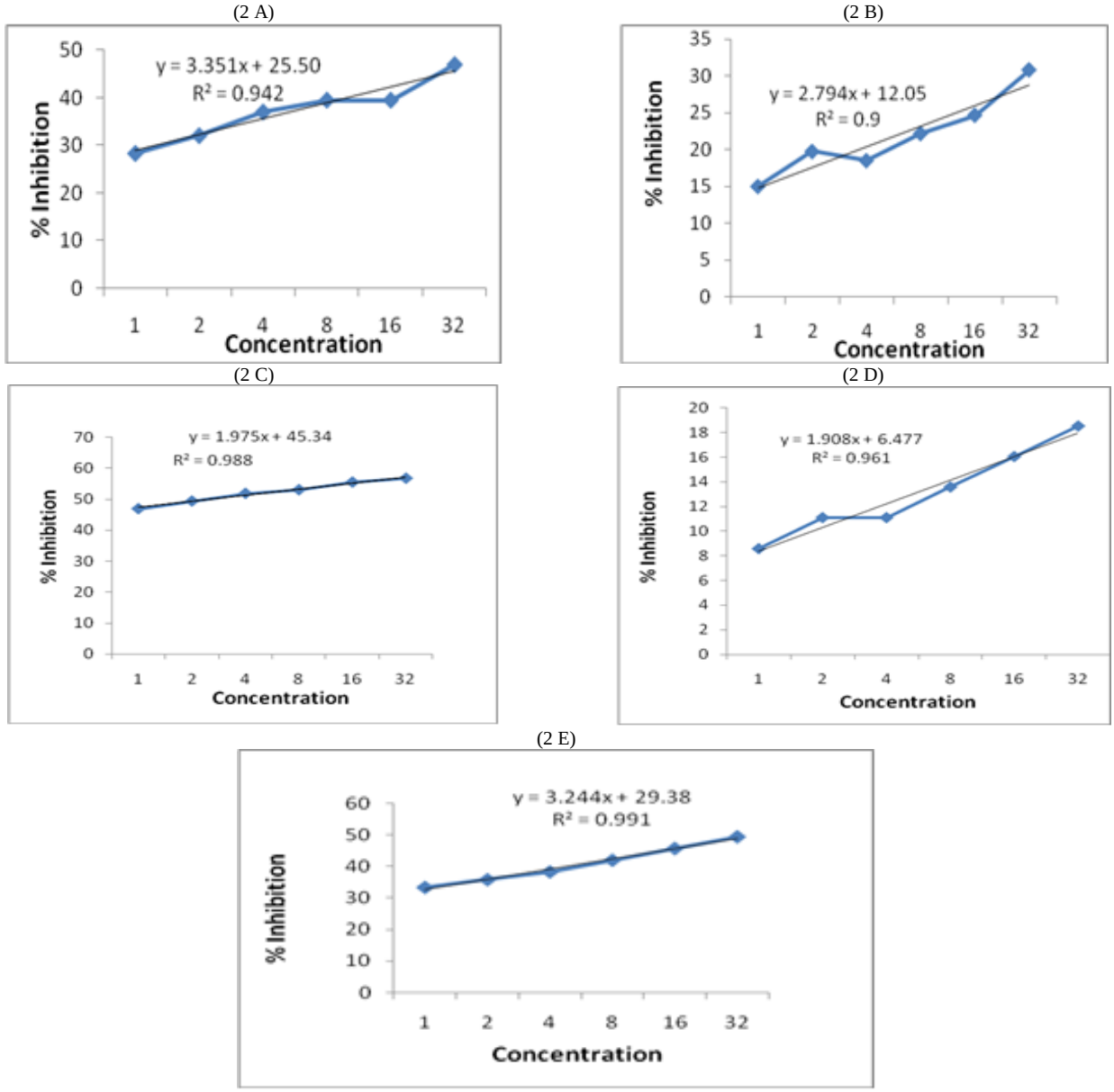


Fig 2: Hydrogen Peroxide Scavenging Assay (2a: Rn Mannose; 2b: Rk Starch; 2c: Rnrnmannose; 2d; Rhrcrmannose; 2e: Ascorbic Acid)

Table 3: IC_{50} Values of Glycopeptides and Ascorbic Acid by Hydrogen Peroxide Scavenging Assay

Compound	IC_{50} (µg/ml)
RN Mannose	7.44
RK Starch	14.07
RNRN Mannose	2.47
RHRCR Mannose	15.2
Ascorbic acid	6.44

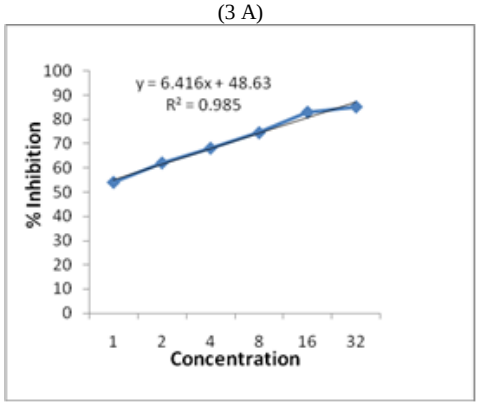
3.2.2. DPPH free radical scavenging assay

In the DPPH free radical method, antioxidant efficiency is measured at ambient temperature and thus eliminates the risk of thermal degradation of the molecules tested. However, the reaction mechanism between the antioxidant and DPPH depends on the structural conformation of the antioxidant (Brand-Williams et al,1995). It was observed that the glycopeptides were capable of neutralizing the DPPH free radicals via hydrogen donating activity at concentrations of 1, 2, 4, 8, 16 and 32 µg/ml, respectively. As shown in Table 4 and Fig. 3, DPPH scavenging activity increased in a concentration dependent manner as compared to ascorbic acid, as the positive antioxidant control in this protocol. IC_{50} values are reported in Table 5.

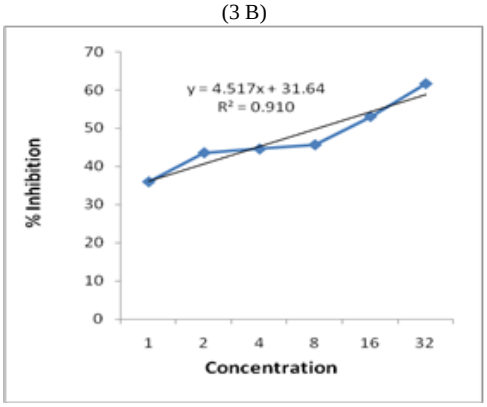
Table 4: Percent Inhibition of Glycopeptides and Ascorbic Acid by DPPH Scavenging Assay

Compound	1µg/ml	2 µg/ml 2	4 µg/ml	8 µg/ml	16 µg/ml	3232 µg/ml
RNMannose	54	62	68.8	74.46	82.9	85.1
RKStarch	36	43.6	44.6	45.7	53.1	61.7
RNRNMannose	48	64	72	85	88	88.8
RHRCR Mannose	35	37	43	47	62	69
Ascorbic acid	52	61	67.02	71.2	74.4	79.7

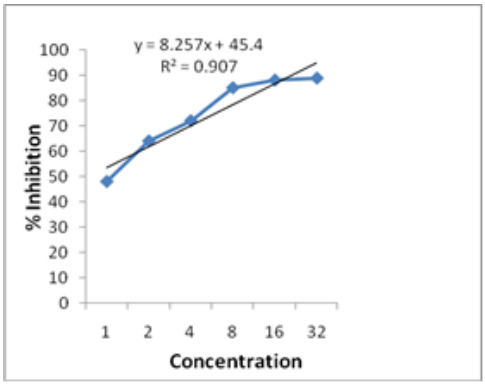
(3 A)



(3 B)

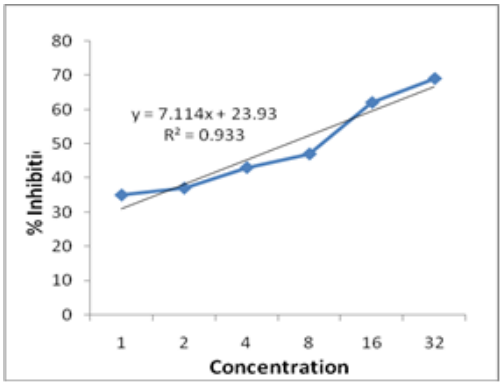


(3 C)



(3 D)

7.2



(3 E)

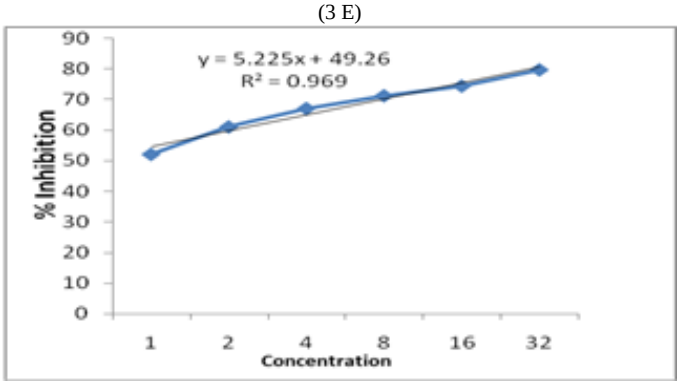


Fig. 3: Dpph Assay (3a: Rnmannose; 3b: Rk Starch; 3c: Rnrn Mannose; 3d: Rhrcr Mannose, 3e: Ascorbic Acid)

Table 5: IC_{50} Values of Glycopeptides and Ascorbic Acid by DPPH Scavenging Assay

Compound	IC_{50} values (µg/ml)
RN Mannose	0.21
1RK Starch	4.08
RNRN Mannose	0.55
RHRCR Mannose	3.67
Ascorbic acid	0.15

3.3.3. Phosphomolybdenum assay

The scavenging ability of different glycopeptides and ascorbic acid was compared and shown in Table 6 and Fig. 4. Ascorbic acid was used as the positive control. The IC_{50} values calculated is shown in Table 7.

Table 6: Percent Inhibition of Glycopeptides and Ascorbic Acid by Phosphomolybednum Assay

Compound	1µg/ml	2 µg/ml 2	4 µg/ml	8 µg/ml	16 µg/ml	3232 µg/ml
RNMannose	33	34.09	35.22	43.18	46.59	55.68
RK Starch	49	54	61	65	72	74
RNRN Mannose	34	38.14	36	42	45	47.42
RHRCR Mannose	42	52	51.5	57.9	62.5	64
Ascorbic acid	38.63	40.9	43.18	50	53.4	57.95

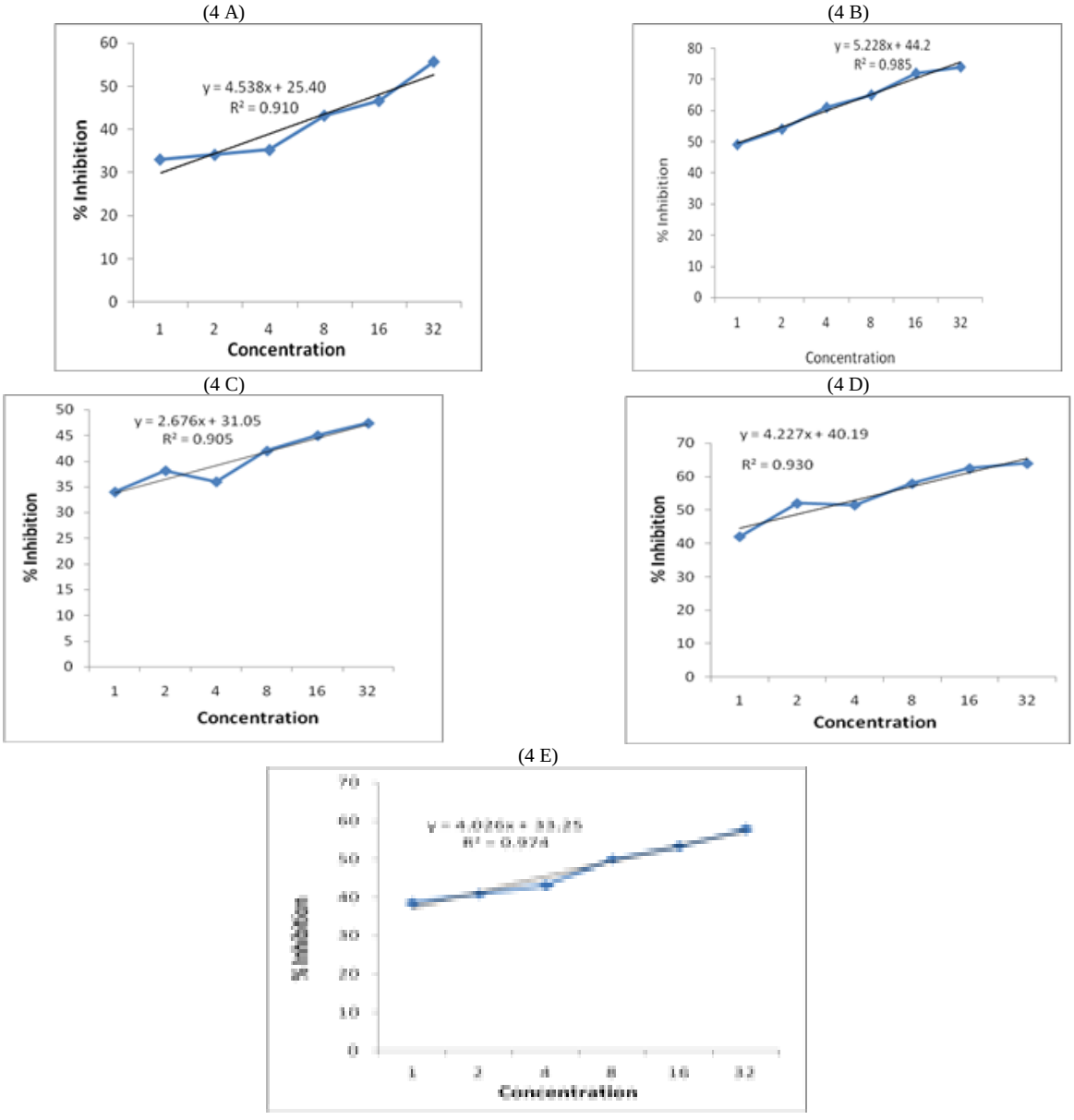


Fig. 4: Phosphomolybednum Assay (4a: Rnmannose; 4b:Rk Starch; 4c: Rnrnmannose; 4d: Rhrcr Mannose; 4e: Ascorbic Acid).

Table 7: IC_{50} Values of Glycopeptides and Ascorbic Acid by Phosphomolybednum Assay

Compound	IC_{50} values (µg/ml)
RN Mannose	5.46
RK Starch	1.11
RNRN Mannose	7.28
RHRCR Mannose	2.35
Ascorbic acid	4.20

4. Conclusion

In the past few years, interest in the search of new antioxidants has grown because reactive oxygen species (ROS) production and oxidative stress is linked to many diseases. Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases [13]. They are also involved in autoimmune disorders like rheumatoid arthritis etc[1], [7]. Therefore, research for the determination of the natural antioxidants source is important.
In this study, we conclude that RN mannose was found to be most potent antioxidant compound where as the swiss dock model used to determine appropriate binding orientations and conformations at binding site with protein shows RHRCR Mannose to have the best affinity for the receptor. This result is contradictory to the results obtained from in-vitro assay method. So, some other docking method may be used to find the exact results of binding.

5. Acknowledgement

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