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NOVEL PEPTIDES IDENTIFIED USING NEXT GENERATION SEQUENCING WITH POTENT ANTI-INFLAMMATORY ACTIVITY

Biological Science

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ABSTRACT

Bioinformatics and Next Generation Sequencing (NGS) advances will give high-throughput molecular data for studying an organism's transcriptome profile and complete genome. *Solanum virginianum* commonly referred to as wild eggplant or nightshade plant is native to India, Australia, and Asia. The Solanaceae plant is used in traditional medicine for its antibacterial, antiviral, antioxidant, hemolytic, and anti-inflammatory properties. In this study, the transcriptome of *S. virginianum* fruit is being studied to identify bioactive peptides. Using *in silico* methods 58 bioactive peptides have been identified of which 13 are anti-inflammatory. Based on the SVM prediction score, two bioactive peptides, SVBP-5 and SVBP-6, have been synthesized and evaluated for antioxidant and anti-inflammatory activities by *in vitro* and *in silico* studies. The different methods employed were DPPH, ABTS, egg albumin, HRBC membrane stabilization, and lipid peroxidation assays. SVBP-5 had the highest IC_{50} values for DPPH and ABTS radical scavenging, with values of 121.3 and 75.93 µg/ml, respectively. Lipid peroxidation was significantly inhibited by SVBP-5. SVBP-5 showed anti-inflammatory properties, with IC_{50} value of 97.8 µg/ml in egg albumin denaturation assay and 123.9 µg/ml in HRBC membrane stabilization. Peptide-protein docking was used to study SVBP-5/6 - human peroxiredoxin 5 (pdb id: IHD2) interactions. Docking studies confirmed the SVBP-5 and -6 pharmacological properties. The found SVBP-5 and -6 may be exploited further as anti-inflammatory molecules.

KEYWORDS

Solanum virginianum, Bioactive peptides, Anti-inflammatory properties.

INTRODUCTION

Next-generation sequencing technology can provide high-throughput resources for whole-genome gene expression profiling [1]. A thorough understanding of an organism's whole genome and transcriptome profile can be attained through next-generation sequencing (NGS) technology and bioinformatics to acquire high-throughput molecular data. Identifying genes primarily in the role of developing pharmacologically active biomolecules or phytocompounds in medicinal plants has been considerably easy by applying genomic technology [2]. Various biological processes were analyzed using bioinformatics techniques to analyze their molecular properties. Bioinformatics, empirical research, and an integrated strategy are some of the crucial phases in identifying bioactive peptides. Applying bioinformatic methods makes it possible to identify if a particular peptide is present in every protein [3].

Additionally, the identified peptides can be synthesized and evaluated for bioactivity. Bioactive peptide purification and separation using standard methods is difficult and time-consuming. Traditional isolation is complex, and assessing the activity quickly is challenging. The fundamental aim of bioactive peptide research is quick and effective isolation. To advance knowledge, Bioinformatics and peptidome methodologies are used in peptide research [4]. Numerous databases have been established to document occurrences of bioactive peptides. Bioactive peptide databases include PepBank, PeptideDB, BIOPEP, APD2, and CAMP. PeptideDB and BIOPEP have identified antibacterial growth factors, peptide hormones, cytokine, and toxin/venom peptides.

However, APD2 and CAMP have concentrated mainly on antimicrobial peptides having antiviral, antifungal, antibacterial, and antiparasitic effects. Databases enable bioinformatics tools to investigate peptides for various purposes. To advance knowledge, Bioinformatics and peptidome methodologies are used in peptide research [5].

The Solanaceae family has garnered significant attention for its agricultural and medicinal significance. It encompasses economically vital plants like potatoes, tomatoes, eggplants, and peppers, making it a crucial subject of study [6]. *Solanum virginianum*, belonging to the Solanaceae *family*, is an annual herb commonly known as "Kantakari." It is also called "Indian nightshade" or "yellow-berried nightshade." This plant is recognized for its diverse medicinal attributes [7]. Plant-derived peptides serve as chemical defenses, safeguarding plants from bacterial pathogens and pests. A thorough literature review has revealed a wealth of bioactive peptides originating from Solanaceae plants, emphasizing their significant presence [8].

Inflammation is a natural self-defense mechanism that our body employs in reaction to harmful stimuli, such as injuries and infections, which have the potential to cause damage to the tissues that are involved. Several biological processes are activated during the inflammatory response. These mechanisms include generating and releasing pro-inflammatory mediators such as cytokines, chemokines, interleukins, and growth factors [9]. The control of inflammatory and cancer conditions frequently involves the administration of small molecules as pharmaceuticals that can effectively interact with a wide range of pharmacological targets. Regrettably, these substances have limitations with high toxicity, little selectivity, and a broad spectrum of undesirable side effects. These include penetrating the brain-blood barrier and producing hazardous compounds during metabolic processes. Additionally, they are frequently insufficient for prolonged therapy. A novel therapeutic approach using bioactive peptides as anti-inflammatory drugs is now under development [10]. Peptides often exhibit greater potency and selectivity towards their targets than small compounds, primarily due to their chemical and resulting biological variety. Various endogenous medicinal peptides, including insulin, have exhibited little toxicity and rapid elimination, while others have proven effective membrane permeability. Therefore, the dosage needed to achieve a therapeutic effect is reduced compared to tiny molecules. The sequence of the peptide heavily influences the efficiency of peptide synthesis. As the complexity of the synthesis process rises, it becomes crucial to perform analytical

characterization, which significantly raises the costs and time needed. Furthermore, they have limited metabolic stability and oral bioavailability unless they undergo chemical alterations and can solely be administered by injection [11,12].

The current study investigates the benefits of *S virginianum* fruit. In this study, we present a novel report on the identified synthesized anti-inflammatory peptides (AIPs), which possess anti-inflammatory properties obtained from the fruit of *S. virginianum* for pharmacological properties. The identification of AIPs has been assisted by using Next-Generation Sequencing (NGS) technology and various bioinformatics tools and evaluation of predicted AMPs: antioxidant properties and anti-inflammatory properties *in vitro* and *in silico*.

MATERIALS AND METHODS

MATERIALS

Chemicals used in this study were methanol, 1,1-diphenyl-2-picrylhydrazyl, 2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid, 2,4,6-Tripyridyl-S-triazine, dimethyl sulfoxide, gallic acid, potassium persulfate, methanol, egg albumin, glacial acetic acid, sodium phosphate, monopotassium phosphate, potassium hydroxide, sodium dodecyl sulfate, thiobarbituric acid, trichloroacetic acid, monosodium phosphate, diclofenac sodium were purchased from sigma, SRL chemicals and SD fine chemicals. All other chemicals and solvents used were of analytical grade.

Transcriptome analysis

Transcriptome analysis was performed using NextSeq500 and 2X 150bp chemistry, 5.31 Gb of high-quality paired-end data was generated, yielding 18,184,076 PE reads and 5,314,463,360 bases. Unigenes were eliminated using CD-HIT-EST-4.6 and Transcoder-v5.3.0 software was used to predict coding sequences from unigenes. CDS were sent to BLAST2Go to predict transcripts with a maximum length. Peptides were predicted using DRAMP IDs with E-values of less than 5, and AMPs with an expected chance score of 0.5 were predicted using AMPA. A peptide with a prediction probability >0.5 was considered as an AMP by the AMP scanner algorithm. Antimicrobial peptides were predicted to come in 58 in number. Most defensins originate from *Brassicaceae*, *Fabaceae*, and *Solanaceae*. Defensins are short (12–45 amino acids), very basic, and have disulphide links between 8–10 cysteines that maintain their stable. To the expected AMPs, defensin peptides were predicted among the projected AMPs [13].

In silico prediction of anti-inflammatory properties of anti-microbial peptides

Anti-inflammatory properties were predicted using preAIP (Prediction of Anti-inflammatory Peptides (kyutech.ac.jp), which is a web-based prediction system for anti-inflammatory properties of antimicrobial peptides, which were predicted using the transcriptome.

Peptide Synthesis

Grey Matter Research Foundation Pvt Ltd in Tamil Nadu, India, developed peptides using prediction tools. Peptides (SVBPs) were synthesized using solid-phase synthesis, purified to >95% purity, the purity was confirmed using mass spectrometry. Stored in 0.01% acetic acid solution at -20°C.

Determination of antioxidant activities of SVBPs

DPPH radical scavenging activity

DPPH radical scavenging activity was performed according to Braca et al [14]. a volume of 50 µl of the sample solution, which had a concentration ranging from 50-300 µg/ml, was mixed with 300 µl of an ethanolic solution containing 0.5 mM DPPH. Following a 5-minute incubation period at a temperature of 37°C in a dark condition, the absorbance of the sample was measured at a wavelength of 517 nm compared to a blank control. The calculation of the radical scavenging activity of SVBPs was performed by determining the percent inhibition using the equation provided.

% Inhibition = [(absorbance control - absorbance test)/ Absorbance control] x100

ABTS radical cation decolorization assay

The ABTS radical cation decolorization experiment was feasible, according to [15]. 2.45 mM potassium persulfate and water containing 7 mM ABTS were mixed together to obtain an ABTS + solution (1:1). This reaction mixture was stored at room temperature in a container for 16 to 20 hours. We prepared an ABTS + solution with an absorbance of 0.7 at 734 nm by diluting it with methanol. Following a 30-minute incubation period, 3.995 ml of ABTS + solution was mixed with 1 ml of different concentrations of synthesized SVBPs (50 to 300 µg/ml), and the absorbance at 734 nm was noted. The observed results were expressed as percentages and were measured as the percent scavenging of SVBPs.

Lipid peroxidation

Lipid peroxidation was measured using thiobarbituric acid reactive substances (TBARS), as reported by Ohkawa et al. [16]. The SVBPs were added to the liver homogenate (10% in cold phosphate buffered saline, pH 7.4) at various concentrations (100–500 µg/ml) in water. The extracts were added into tubes with acetic acid (20 % v/v, pH 3.5), SDS (8 % w/v, 0.2 ml), and thiobarbituric acid (0.8 % w/v, 1.5 mL). For forty-five minutes, the mixture was kept in a hot water bath. Three milliliters of 1-butanol were used to extract the TBARS that were produced, and the samples were measured at 532 nm using L-ascorbic acid as the standard.

Determination of the in vitro anti-inflammatory activity of SVBPs

Egg albumin denaturation test

According to Gupta et al. [16], The *in vitro* anti-inflammatory properties of SVBPs were evaluated using the egg albumin denaturation assay. 0.2 mL of egg albumin, 2 mL of five different SVBPs concentrations (10 to 300 µg/mL), and 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) were mixed together to create a reaction mixture. An equivalent volume of distilled water was utilized as a control. Following a 15-minute incubation period at 37 °C, the mixtures were heated for 5 minutes at 70 °C. After cooling, a blank solution was used to measure absorbance at 660 nm. Diclofenac sodium was used as a control medication, and its absorbance was evaluated under the same conditions at final concentrations of 10, 50, 100, 150, and 200 µg/mL. The procedure was carried out three times. The % inhibition of protein denaturation was calculated using the following formula.

$$\% \text{ inhibition} = [\text{Acontrol} - \text{Asample} / \text{Acontrol}] \times 100$$

Membrane stabilization test

A centrifuge tube containing human blood was treated with 2 mg of disodium EDTA in order to avoid the Human Red Blood Cell (HRBC) Suspension from clotting during preparation. The blood was centrifuged at 1000 rpm for 10 minutes in order to remove the erythrocyte particles. After carefully resuspending the pellet to obtain a 10% (v/v) suspension, the particles were centrifuged at 1000 rpm for 10 min in pyrogen-free saline (0.9% NaCl) and suspended in normal saline.

Hypotonicity-induced hemolysis

HRBC membrane stabilization of SVBPs was performed by method [17]. The synthesized SVBPs were dissolved in hypotonic distilled water and isotonic phosphate buffer solution to obtain concentrations between 10 to 300 µg/mL. Five milliliters of distilled water were added into test tubes together with diclofenac sodium, which had been dissolved at the necessary concentration in an isotonic phosphate buffer solution. This served as a positive control. After each test vial had received 0.1 mL of erythrocyte suspension, it was gently shaken. The test containers were centrifuged for five minutes at 3000 rpm after being incubated for thirty minutes at 37 °C in a water bath. A UV spectrophotometer set at 540 nm was used to measure the supernatant in order to calculate the amount of hemoglobin released through hemolysis. The percentage of hemolysis was determined under the condition that all hemolysis produced in the presence of distilled water was 100%. To calculate the percentage of hemolysis inhibition by the SVBPs, an equation was utilized.

$$\% \text{ Inhibition of Hemolysis} = [1 - \text{OD2} - \text{OD1} / \text{OD3} - \text{OD1}] \times 100$$

where OD1, OD2, and OD3 are the absorbance of the test sample in an isotonic solution, the absorbance of the test sample in a hypotonic solution, and the absorbance of the control sample in a hypotonic solution, respectively.

Molecular docking studies

Evidence on binding conformation, pattern, and affinity can be found *in silico* molecular docking studies of chemical drug compounds or SVBPs that work by interacting with receptors. The protein was obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (http://www. rcsb.

org/pdb) and assigned with proper three - dimension orientation. SVBPs were converted to a PDB file by using the PREPFOLD server. The energy-minimized protein was then used as input for HPEPDOCK SERVER to carry out the docking simulations. Human peroxiredoxin 5 *(*pdb id*: IHD2)* [16] was obtained from the RCSB PDB they were used as receptor molecules. Before analysis, water molecules and other unwanted residues were removed from all proteins, when necessary, using Discovery studio software. The sequences were then subjected to energy minimization by Swiss-PdbViewer v4.1.0. The docking algorithm provided with HPEPDOCK was used to search for the best-docked conformation between ligand and protein. During the Docking process, a maximum of 15 conformers were considered for ligand. Discovery Studio software was used to deduce the 2D and 3D pretorial representation of the interaction between the peptides and receptors.

Statistical Analysis

The collected data of the results were analyzed statistically and reported as mean ± SD using Prism Pad software (Version 5.0) (Graph Pad Software, Inc., San Diego, CA, USA).

RESULTS

The research study focused on the evaluation of AIPs (anti-inflammatory peptides) that were identified and synthesized from the transcriptome of *S. virginianum* fruit. The objective of this study was to assess the potential of peptides that exhibit antioxidant and anti-inflammatory properties. The results of the study indicated the presence of 58 (SVBPs, among which 13 were identified as having potential anti-inflammatory properties. Among these peptides, two were chosen for synthesis based on the SVM score prediction.

DPPH radical scavenging activity

The SVBPs radical scavenging properties by measuring changes in the absorbance of the DPPH at 517nm was done. The DPPH radicals were scavenged by two peptides in a dose-dependent manner. Antioxidant activity of SVBPs was measured by the DPPH method. IC_{50} of the SVBP-5, SVBP-6 and ascorbic acid were 121.3 µg/ml, 168.7 µg/ml and 106.6 µg/ml respectively (Figure 1). The SVBP-2 showed maximum radical scavenging activity compared to the SVBP-6. The % inhibition of free radical (DPPH) scavenging activity at 300 µg/mL of concentration. Lee et al. conducted a study on the characterization of antioxidative peptide purified from black eelpout (*Lycodes diapterus*) hydrolysate. The DPPH radical scavenging activity of black eelpout hydrolysates was used to assess their antioxidant content.

ABTS radical cation decolorization assay

Antioxidant activity of SVBPs was measured by the ABTS method. All SVBPs scavenged the ABTS radical in a concentration-dependent manner (10-500 µg/mL) (Figure 2). IC_{50} of the SVBP-5, SVBP-6 and ascorbic acid were 75.93 µg/ml, 78.71 µg/ml and 60.86 µg/ml respectively. The SVBP-5 exhibited more activity as a radical scavenger than the SVBP-6.

Lipid peroxidation

Inhibition of lipid peroxidation of SVBPs was measured by the thiobarbituric acid reactive substances (TBARS) method. Figure 3 depicts the inhibitory action of SVBPs was determined at concentrations of 100 – 500 µg/mL. IC_{50} of the SVBP-5, SVBP-6 and ascorbic acid were 240.5 µg/ml, 255.4 µg/ml and 226.0 µg/ml respectively. The SVBP-5 exhibited more activity as a lipid peroxidation inhibitor than the SVBP-6.

Egg albumin denaturation assay

In this study, we aimed to investigate the potential anti-inflammatory effects of SVBPs on *in vitro* denatured egg albumin. The results indicated that SVBPs were able to suppress protein denaturation in a concentration-dependent manner, with mean suppression values ranging from 10 to 300 µg/mL (Figure. 4). IC_{50} of the SVBP-5, SVBP-6 and ascorbic acid were 97.18 µg/ml, 106.5 µg/ml and 82.83 µg/ml respectively. The SVBP-5 exhibited more activity as anti-inflammatory property than the SVBP-6.

Membrane stabilization test by hypotonicity-induced hemolysis

The HRBC technique, which includes stabilizing the membrane, is an excellent approach for evaluating anti-inflammatory effects *in vitro*. By stabilizing the membrane of the red blood cell (RBC), which is similar to the membrane of the lysosome, the test sample's ability to maintain lysosomal membranes is also shown. Figure 5 illustrates the potent anti-inflammatory effects of SVBPs. Percentage of inhibition hemolysis of the SVBP-5, SVBP-6 and ascorbic acid were 123.9 µg/ml, 134.4 µg/ml and 116.9 µg/ml respectively. The SVBP-5 exhibited more activity as anti-inflammatory property than the SVBP-6.

Molecular docking studies

The application of computational docking studies has shown its efficacy in the fields of drug discovery and the development of small molecule drugs [20]. The study carried out involved subjecting the SVBPs to protein-peptide docking studies using the HPEPDOCK server. The *human* peroxiredoxin 5 *(*pdb id*: IHD2)* was employed for this purpose of docking studies with SVBPs. The studies of the docking investigation suggested that SVBPs had significant binding modes, and they showed an excellent binding energy score with their respective anti-inflammatory target protein. This was confirmed by the fact that the bioactive peptides formed stable complexes with the target protein. SVBP-5, one of the SVBPs, was shown to have the highest binding affinity with the human peroxiredoxin 5 *(*pdb id*: IHD2)* (Figure 6, Table 1).

DISCUSSION

Inflammation is recognized as a component of the normal host response to injury or illness. The human immunological and inflammatory response is regulated by a highly intricate set of variables [21]. The inflammatory process initiates the immune response to poisons, invading microorganisms, allergens, and injured tissue. Inflammatory responses can be triggered by immunological, microbiological, and toxic stimuli that stimulate different types of cellular and humoral components [22]. Inflammatory stimuli, such as molecules from pathogens, damaged cell products, toxins, or allergens, initiate inflammatory reactions. These reactions result in the release of cell-derived mediators, including cytokines from the interleukin (IL) families, tumor necrosis factor-alpha (TNF-α), prostaglandins (PG), nitric oxide (NO), and leukotrienes (LTs). During the first stage of inflammation, there is a significant release of lipid mediators that can have either pro-inflammatory or anti-inflammatory effects. Studies have indicated that arachidonic acid (AA)-derived lipid mediators, known as eicosanoids, are linked to several stages of the inflammatory process. Lipids can be liberated from phospholipids in the cell membrane during inflammation and broken down into prostaglandins (PGs) and leukotrienes (LTs) [23,24]. Although there have been recent advancements, the current treatment methods remain insufficient for addressing organ failure, shock, and sepsis, which are significant obstacles in the field of intensive-care medicine (ICU). Therefore, managing inflammatory illnesses and various components of the complex inflammatory responses continues to be an essential responsibility. The extensive range of immunomodulatory capabilities ascribed to anti-inflammatory peptides (AIPs), such as their ability to regulate immune cell development and inhibit excessive pro-inflammatory reactions, including sepsis and allergies, has garnered considerable interest. Furthermore, AIPs have been found to possess other immunomodulatory properties, including the capacity to enhance wound healing, eliminate microbes, and promote angiogenesis [25,26,27,28].

Recently, several anti-inflammatory peptides (AIPs) have been used as pharmaceuticals to prevent and manage inflammation. In the case of rheumatoid arthritis, it has been discovered that the presence of vasoactive intestinal peptide (VIP) can effectively reduce both inflammation and the body's inflammatory response. Several additional AIPs have demonstrated antioxidant effects and the ability to inhibit the generation of reactive oxygen species (ROS), which are involved in developing several chronic and acute inflammatory conditions [29,30].

SVBPs exhibited the DPPH and ABTS scavenging ability and restored the level of lipid peroxidation. Similar to our study lee et al studied the DPPH activity of antioxidative peptide purified from black eelpout (*Lycodes diapterus*) hydrolysate [31]. However, jiang et al reported the ABTS activity of tripeptides isolated from fermented grains (*Jiupei*) and the antioxidative interaction with 4،methylguaiacol, 4،ethylguaiacol, and vanillin [32] and xue et al. reported the malonyldialdehyde (MDA) of three peptide fractions of Rapeseed (*Brassica napus*) Protein Hydrolysate [33].

Inflammation arises when triggering factors stimulate inflammatory pathways, leading to the release of inflammatory factors [34]. Inflammatory factors, such as interleukin-β1 (IL-β1), interleukin-6

(IL-6), and tumor necrosis factor-α (TNF-α), play a crucial role to promote inflammation in conditions such as inflammatory bowel disease (IBD), obesity, and other inflammatory disorders [35]. SVBPs showed the significant anti-inflammatory properties both *in vitro* and *in silico*. Similar to our study, Derbel et al. studied the in vitro antioxidant and anti-inflammatory activities of bioactive proteins and peptides from *Rhodomonas* sp [36]. Whereas Mudgil et al. studied the molecular binding mechanism and identification of novel anti-hypertensive and anti-inflammatory bioactive peptides from camel milk protein hydrolysates [37]. SVBPs showed the significant binding energy with *human* peroxiredoxin 5 *(*pdb id*: IHD2)*.

CONCLUSION

In conclusion, anti-inflammatory peptides were identified through transcriptome analysis of the whole fruit of *Solanum virginianum*. These SVBPs were synthesized using solid-phase synthesis. The study aimed to analyze SVBPs for their pharmaceutical properties, particularly antioxidant and anti-inflammatory activities. SVBPs exhibited antioxidant properties by scavenging DPPH and ABTS free radicals. Furthermore, they displayed anti-inflammatory effects without toxicity. These findings highlight SVBPs' potential as versatile peptides with applications in pharmaceuticals. SVBPs show promise in controlling and treating oxidative stress-triggered metabolic and inflammatory diseases. Investigating SVBPs' molecular mechanism in relation to oxidative stress holds significant potential for novel treatments.

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Data availability statement:

Author contribution

Megha G T: Concept, design, literature survey, sample preparation, experimental studies, data acquisition and analysis, statistical analysis, manuscript preparation and review. Ravikumar H: experimental studies, data acquisition and analysis, statistical analysis. Manjunatha D: experimental studies, data acquisition and analysis, statistical analysis. Thoyajakshi R S: Sample collection, experimental studies, data acquisition and analysis, statistical analysis. Rajeshwar Achur A N: Conceptualization, writing, supervision, project administration. Nagaraju S: Conceptualization, clinical and experimental design, writing-original draft, manuscript preparation and review, supervision, project administration.

Competing interests

The authors have no conflicts to disclose

Acknowledgement

Not applicable.

Table and figures:

Table 1: Docking scores of peptide-protein complexes of SVBPs

Target protein	Docking score(kcal/mol)		
	SVBP-5	SVBP-6	Standard Citropin 1.1
Human peroxiredoxin 5 (pdb id: *IHD2*)	-185.309	-161.72	-174.03

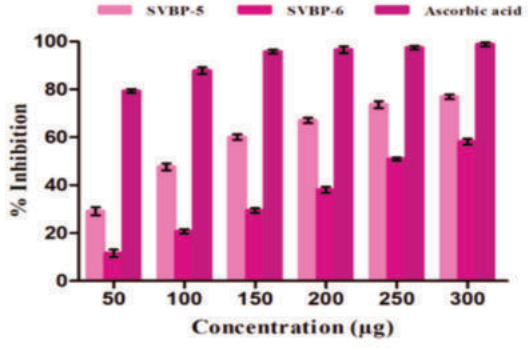

Figure 1. 1,1-Diphenyl-2-picrylhydrazyl free radical scavenging activity. Antioxidant activity of SVBPs were measured by the DPPH method. IC_{50} of the SVBP-5 and SVBP-6 were 121.3 µg/ml and 168.7 µg/ml respectively whereas the IC_{50} value of the standard ascorbic acid was 106.6 µg/ml. Each value is represented as mean ± SD of three independent experiments.

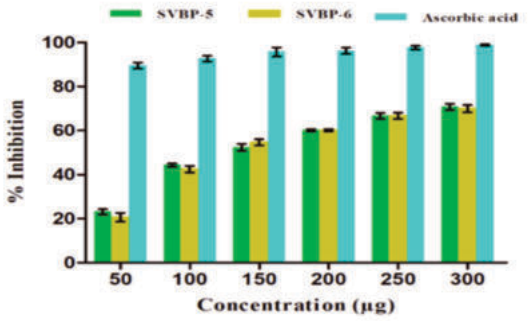

Figure 2. ABTS radical cation decolorization assay Antioxidant activity of SVBPs were measured by the ABTS method. IC_{50} of the SVBP-5 and SVBP-6 were 75.93 µg/ml and 78.71 µg/ml respectively whereas the IC_{50} value of the standard ascorbic acid was 62.86 µg/ml. Each value is represented as mean ± SD of three independent experiments.

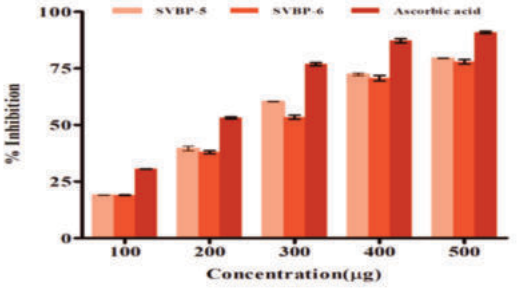

Figure 3. Inhibition of lipid peroxidaton. The Inhibition of lipid peroxidaton of SVBPs were measured by the TBARS method. IC_{50} of the SVBP-5 and SVBP-6 were 240.5 µg/ml and 255.4 µg/ml respectively whereas the IC_{50} value of the standard ascorbic acid was 226 µg/ml. Each value is represented as mean ± SD of three independent experiments.

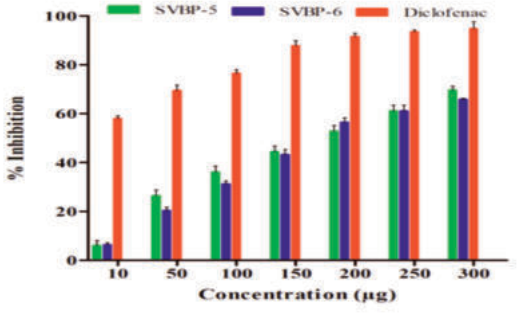

Figure 4. Egg albumin denaturation assay. Percentage inhibition of SVBPs and diclofenac against egg albumin denaturation. IC_{50} of the SVBP-5 and SVBP-6 were 97.18 µg/ml and 106.5 µg/ml respectively whereas the IC_{50} value of the standard diclofenac was 82.83 µg/ml. Each value is represented as mean ± SD of three independent experiments.

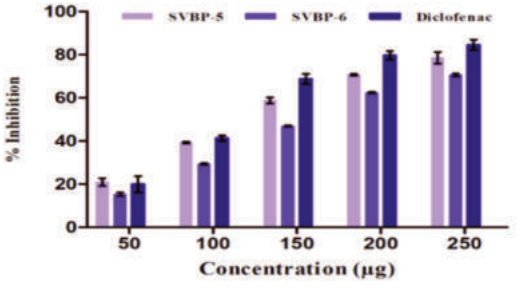

Figure 5. HRBC membrane stabilization assay. Percentage of

membrane stabilization of hypotonicity induced hemolysis. IC_{50} of the SVBP-5 and SVBP-6 were 123.9 µg/ml and 134.4 µg/ml respectively whereas the IC_{50} value of the standard diclofenac was 116.0 µg/ml. Each value is represented as mean ± SD of three independent experiments.

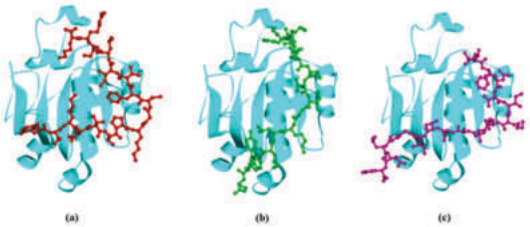

Figure 6: Molecular docking conformation of Peptide – protein docking Docking studies and interactions for peptide-protein complexes of peptides and Human peroxiredoxin 5 (pdb id: *IHD2*) (a)Peptide- protein complex of SVBP-5 with Human peroxiredoxin 5 (pdb id: *IHD2*). (b) Peptide- protein complex of SVBP-6 with Human peroxiredoxin 5 (pdb id: *IHD2*) (c) Peptide- protein complex of Standard Citropin 1.1 with Human peroxiredoxin 5 (pdb id: *IHD2*).

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