Bharath
INSTITUTE OF HIGHER EDUCATION AND RESEARCH
(Declared as Deemed-to-be University under section 3 of UGC Act, 1956)
(Vide Notification No. F.9-5/2000 - U.3, Ministry of Human Resource Development, Govt. of India, dated 4th July 2002)
Accredited by NAAC NATIONAL ASSESSMENT AND ACCREDITATION COUNCIL
Phone : 044-22290742 / 22290125 . Telefax : 044-22293886
Website : www.bharathuniv.ac.in
173, Agaram Road, Selaiyur, Tambaram,
Chennai - 600 073. Tamil Nadu.

Ref. No.SMS-2018-O-03

Date: 29.08.2019

TO

Mr. R. Vijaykumar
Professor/Physiology
BIHER.



Thro: Concern Head of the Department

Greetings!!!

We are happy to announce that the Research Advisory Committee has approved your proposal for Seed Money Scheme-2018 which was presented by you. You are requested to complete the proposal and send the progress report to the Dean Research in the prescribed time period.

**Title of the Project: In Vitro and In Silico Screening and Characterization of Antimicrobial Napin Bioactive Protein in Brassica juncea and Moringa oleifera**

**Seed Money Amount: Rs.1, 00,000/- (Rupees One Lakh Only)**

**Approved on: 22.08.2019**

**Payment details:**

**Voucher No.58** **Dated: 03.09.2019**

With Regards

Dean-Research

# Bharath University

SELAIYUR, CHENNAI - 600 073, TAMIL NADU, INDIA.

## CASH / PAYMENT VOUCHER

Date 03/09/2019

V.No. 58

Debit _____ Amount

**Rs.** 1,00,000/-

PAID TO Dr. R. Vijay Kumar

RUPEES One lakh only

TOWARDS Seed Money Scheme-2018

BHARATH UNIVERSITY CHENNAI 600 073. INDIA.

**Authorised by** **Finance Manager** **Cashier/Accountant** **Payee's Signature**

# PROPOSAL SUBMISSION

1. **Details of Principal Investigator**


| | |
|---|---|
| **Name** | : Dr.R.Vijayakumar |
| **Designation** | : Professor |
| **Highest Qualifications** | : Ph.D. |
| **Department** | : Physiology |
| **E-mail** | : **sivanviji@gmail.com** |
| **Contact no** | : 9445383846 |
| **Date of Joining** | : 02.01.2012 |


2. **Details of Principal Investigator**


| | |
|---|---|
| **Name** | : Dr. S.Latha |
| **Designation** | : Associate Professor |
| **Highest Qualifications** | : Ph.D. |
| **Department** | : Physiology |
| **E-mail** | : **lathaviji.kumar@gmail.com** |
| **Contact no** | : 9445451480 |
| **Date of Joining** | : 02.01.2014 |

## Technical details

### 1. Introduction:

Water scarcity has been predicted globally and particularly in India by 2020 [1]. The major problem is poor water quality, and it is estimated that waterborne diseases affect about 37.7 million Indians annually and 1.5 million children under the age of five die due to diarrhea each year [2]. A major portion of the rural population depends mostly on groundwater, whereas the urban population depends on surface water. Increased pressure on the resources due to the alarmingly growing population and other factors such as industrial discharge, agricultural run-off, and poor sanitation practices put the long-term availability and quality of the potable water at stake. In most developing countries, including India, farming by-products, crop residues, and grazing along with some protein and energy supplements are the main source of livestock feed for ruminant animals. Common protein supplements for ruminants are oilseed cakes obtained as oil industry by-products. Mustard (Brassica juncea) and Moringa (Moringa oleifera) cake is the most widely available protein substitute for livestock in Asian countries [3]. Microbial contamination through fecal contamination in water is the major reason for the poor water quality in developing countries, transmitting a large number of diseases. The common pathogens present in the drinking water include Shigella species, Salmonella species, Klebsiella species, Escherichia coli, Enterobacter species, and parasites such as Giardia lamblia and Entamaebahistolytica [4, 5]. Drinking water treatment involves several combined processes based on the quality of the water source and the cost and availability of chemicals in achieving the desired level of treatment and water quality standard as recommended by the World Health Organization (WHO) [6]. The available water treatment processes are expensive, especially in developing countries. Synthetic organic and inorganic chemicals are commonly used for various water treatment processes and are associated with environmental and human health problems [7]. As an alternative, natural materials (plant material) can be used for water treatment. Moringa (Moringa oleifera) is one of the widely cultivated species in the tropical region of Asia, Africa, and South America, and it is used in rural areas of Africa for water treatment [8]. The previous study indicates that various parts of Moringa such as leaves, roots, and bark have antibacterial activity, and the seeds are well known for their coagulant and antibacterial properties [9–15]. The seed extract and recombinant protein of M. oleifera are effective against Staphylococcus aureus, Streptococcus pyrogenes, Streptococcus mitis, Streptococcus pneumoniae, Enterococcus faecalis, Escherichia coli, and Legionella pneumophila for up to 150 min [15]. Although Moringa leaf extract has been extensively studied to be a potent coagulant and antimicrobial agent, there are some concerns such as (i) the availability of Moringa leaf and cost, (ii) the difference in the coagulation property of leaf extracts collected from different localities, and (iii) the type and quality of surface water (presence of contaminants, physical and chemical properties) to be treated. A screening study was conducted to find out coagulant protein from plant materials as a complement to Moringa seeds for water treatment in Southern India [16]. The Yellow (Sinapis alba) and Brown (Brassica juncea) mustards possess glucosinolates. These glucosinolates and their breakdown products, such as isothiocyanates (ITC) and allylisothiocyanate (AITC) subsidize their natural antimicrobial activity. ITC and particularly purified AITC have been widely studied as antimicrobials and have a broad antimicrobial range

inhibiting Gram-positive and Gram-negative bacteria, yeasts, and molds [17]. The seed extract of Mustard species (Brassica family) was identified to possess coagulation activity against synthetic clay and turbid pond water that can act as a potential natural water treatment agent [13, 18–20] and can also be a good compliment to Moringa coagulant protein. The antimicrobial activity of Mustard leaf extract is not yet explored. The current study aimed to investigate the antibacterial effect of Moringa and Mustard leaf extracts and antimicrobial-coagulant protein against thirteen different clinical pathogens isolated from patients samples in India. The Moringa and Mustard leaf were collected in Tamilnadu, Southern India. The coagulant protein was separated by spin column chromatography, and based on LCMS, the protein sequences were identified; the effect of crude extract and coagulant protein on bacterial cell aggregation study was performed by microscopic observation and growth inhibition assay, and the mode of action of the antimicrobial peptide against Gram-negative pathogens was determined by turbidity measurement in a spectrophotometer. Furthermore, the mode of action was determined by applying the simulation docking model.

## 2. Review of status of Research and Development in the subject

Silva, A.K.; Christofaro, D.G.; Vanderlei, F.M.; Barbosa, M.P.; Garner, D.M.; Vanderlei, L.C. Association of cardiac autonomic modulation with physical and clinical features of young people with type 1 diabetes. Cardiol. Young 2017, 27, 37–45

The presence of risk factors is associated with negative modifications in the autonomic behavior in individuals with type 1 diabetes. Colhoun et al. [10] studied 160 adults with type 1 diabetes, and found an inverse association between global variability and several other factors, such as age, disease duration, higher blood pressure, body mass index, waist/hip ratio, triglycerides, HbA 1c, and physical activity. Similar data were also provided by the EURODIAB Prospective Complications Study Group [11], who highlighted HbA1c, hypertension, distal symmetrical polyneuropathy, and retinopathy as factors capable of predicting the risk of cardiac autonomic dysfunction in type 1 diabetics in a period of 7.3 years

### 2.1. International Status:

The goals of this study were 1) to evaluate HRV in TODAY participants compared with an obese control group and establish the prevalence of cardiac autonomic dysfunction in the TODAY cohort at follow-up (T2P1); 2) to determine whether prior T2D treatment assignment in TODAY (metformin alone, metformin + rosiglitazone, or metformin + intensive lifestyle intervention) or glycemic control over time are independently associated with impaired HRV; and 3) to assess of the association of cardiac autonomic function with noninvasive measures of arterial stiffness.

### 2.2. National Status:

**NIL**

**3. Progress/ achievement so far, if any**

a). Reference papers was collected.
b). Literature survey was studied.
c). Materials and methods were designed.

**4. Work plan**

**4.1 Methodology**

**4.1. Materials and Chemicals**

The following materials, chemicals, solvents, and drugs were used: Paper Disc (Advantec, India), Disk Diffusion Zone (Inhibitor) measuring ruler (Himedia, Chennai, India), Ethanol, Methanol (Fisher Scientific, Chennai, India), 96% Ethanol (Fisher Scientific, Gangnam, Seoul, South Korea), Mueller–Hinton Agar (Himedia, Chennai, India), Nutrient Broth (Himedia, Chennai, India), Mannitol salt agar (Himedia, Chennai, India), Triptic soy agar Phosphate buffer Saline (Fisher Scientific, Chennai, India), Antibiotics (ampicillin, vancomycin, penicillin, gentamicin, tetracycline, kanamycin, clindamycin, erythromycinn, and novobiocin (Himedia, Chennai, India).

**4.2. Isolation of Pathogenic Microorganisms from Patient's Sample**

The clinical samples such as urine, blood, stool, throat swab, and sputum were rolled on the culture plates (Nutrient Agar, Blood Agar, MacConkey Agar, and Chocolate Agar) for the isolation of Pathogenic Microbes, which were incubated overnight and examined for the growth. If there was no growth in 24 h, the plates were incubated further. Organisms were identified based on biochemical reactions (Coagulase, Indole, Citrate, Urease, Mannitol Motility, Triple Sugar Iron Agar, and Phenyl Alanine Deaminase) and colony characteristics.

**4.3. Microbial Strains Applied in the Study**

Clinical isolates of thirteen bacterial strains—Escherichia coli (E.coli), Salmonella paratyphi B (S. paratyphi B), Enterobacter species (E. species), Shigella flexneri (S. flexneri), Salmonella typhimurium (S. typhimurium), Salmonella typhi (S. typhi), Salmonella aratyphi A (S. paratyphi A), Klebsiella pneumonia (K. pneumonia), Staphylococcus aureus (S. aureus), Serratia marcescens (S. marcescens), Shigella dysentriae (S. dysentriae), Proteus mirbalis (P. mirbalis), and Enterococcus faecalis (E. faecalis)-were obtained from Department of Microbiology, Ramachandra Hospital in Chennai, India. Stock culture was maintained in nutrient agar media at 4 C.

**4.4. Extraction of Crude Protein (Active Compounds) from Plant Leaf**

Moringa oleifera (MOS) and Mustard Brassica juncea (BJS) leaf were purchased from local shops in Southern India. The preparation of crude extracts from Moringa and Mustard leafs were

performed as described earlier [19]. Dried MOS and BJS were grounded into fine powder by using mortar and pestle. To remove oil from the fine powder, 95% ethanol was added, and the supernatant was separated by centrifugation at 3000 rpm for 10 min. The pellet was allowed to air dry, and 5% aqueous extract was prepared using sterile distilled water. The obtained soluble fraction is referred to as crude extract (CE).

### 4.5. Bioactive Protein-Functional Activity

#### 4.5.1. Coagulation Activity

The crude extracts and the coagulant proteins were tested for coagulation activity using synthetic clay solution as described earlier [11]. Clay solution (1%) was prepared using kaolin clay and mixed with protein to get a final volume of 1 mL and initial optical density at 500 nm and after 60 min was measured. The percentage of coagulation activity was calculated using the formula: (Coagulation activity % = [(Initial absorbance / finalabsorbance) / initial absorbance] /100).

#### 4.5.2. In Vitro Assay for Antimicrobial Activity Disc Diffusion Kirby Bauer Method

The antagonistic effect of Moringa oleifera crude protein (MOS) 0.016, 0.02, 0.03, 0.04, and 0.08 mg/mL; Brassica juncea crude protein (BJS) 0.017, 0.02, and 0.05 mg/mL; Moringa oleifera antimicrobial-coagulant Protein (MCP) 0.006, 0.012, 0.015, and 0.03 mg/mL, and Brassica juncea antimicrobial-coagulant Protein (BJP) 0.001, 0.015, 0.02, and 0.05 mg/mL was identified using selected enteropathogens acquired from Department Food Science and Biotechnology, Anna University, India. The clinically isolated pathogenic strains were applied to an antimicrobial activity measuring disk that was 8 mm in diameter. The spread plate method was used to inoculate each microorganism on nutrient agar plates. The plates were incubated at 37

#### Minimum Bactericidal Activity (MBC)

The clinical isolates were grown in nutrient broth (NB) and incubated at 37C in a shaking incubator for overnight and diluted to an initial optical density (OD) of 0.1 and 0.3 at 600 nm for growth inhibition assay and cell aggregation test, respectively. The concentrations of the protein that were used for cell aggregation tests were MOS 0.016, 0.02, 0.03, 0.04, and 0.08 mg/mL; BJS 0.017, 0.02, and 0.05 mg/mL; MCP 0.006, 0.012, 0.015, and 0.03 mg/mL and Mus CP 0.001, 0.015, 0.02, and 0.05 mg/mL. Furthermore, the CE and CP were added to the culture suspension with an initial OD of around 0.3 and incubated at 37 or 4 h. The cell aggregation was observed at each hour under the phase-contrast microscope (Nikon Eclipse 80i, Japan), and the images were captured using a microscopic digital camera (EM- 200F, USB-2.0, CCD Chip). The growth kinetics studies were conducted by adding different concentrations of protein to pre-diluted overnight cultures (0.1 OD) to a final volume of 1 mL in a sterile cuvette and incubated at 37 C for 4–6 h with continuous shaking. The OD at 600 nm was measured every 60 min by spectrophotometer (Eppendorf) and values were recorded [51].

### 4.5.3. Electron Microscopy Analysis

Bacterial cells were set at 30 ± 2 for 1 h with 2% glutaraldehyde and paraformaldehyde. After many washes with 0.1 M cacodylate buffer, cells were dehydrated with ethanol [51]. Then, bacterial cells were injected with increasingly concentrated Eponate 812 and then polymerized at 60 ± 2 C for 2 days. Sectioned with ultra-microtome and stained with uranyl acetate using field-emission transmission electron microscope (FETEM) (JEM-2100F, JEOL) KBSI, Chuncheon, Gangwon-do, Korea.

## 4.6. Purification of Coagulant and Antimicrobial Protein

### 4.6.1. Purification of Protein Based on Column Chromatography

Based on size-exclusion spin column chromatography, a < 30 KDa spin column with < 3 KDa filtrate was subsequently applied to separate the low molecular weight (< 3 KDa) antimicrobial peptides (AMPs).

### 4.6.2. Tris-Tricine SDS-PAGE Electrophoresis and Gel-Elution

Furthermore, Tris-Tricine SDS-PAGE (polyacrylamide gel electrophoresis) [21] was applied to separate the <10 KDa peptide band, and based on gel elution, a <3 KDa peptide band was eluted and proceeded for LC-ESI-TOF-MS/MS (mass spectrometry liquid chromatography electrospray ionization-quantitative time-of-flight tandem mass spectrometry).

### 4.6.3. Quantification of Protein Concentration

The protein content of MOS and BJS crude extracts and coagulant protein was estimated by Lowry's method [19]. The purity of the coagulant protein was analyzed by Tris-Tricine SDS-PAGE gel analysis according to Laemmli [20]. The gels were stained with Coomassie brilliant blue to visualize the protein.

### 4.6.4. Identification of Purified Peptides by Mass Spectrometry

Sequential identification of peptides by LC-ESI-TOF-MS/MS (mass spectrometry liquid chromatography-electrospray ionization-quantitative time-of-flight tandem mass spectrometry) were analyzed at the National Instrumentation Center for Environmental Management of Seoul National University in Seoul, Korea, according to an earlier method by Chelliah et al. [51]. The peptides were inspected using QS 3.0 software (Applied Biosystems, Seoul, Korea). The ranges of proteins were identified based on 300–3000 m/z values.

**4.7. SWISS-MODEL: Homology Modeling of Protein Structures:** The protein models were spawned by SWISS-MODEL, licensed under the Creative Commons Attribution-Share Alike 4.0 (CC BY-SA 4.0) International License, based on the sequence generated by liquid chromatography-mass spectrometry (LCMS). SWISS-MODEL generates theoretical models by automated homology modeling techniques developed by the Computational Structural Biology

Group at the Swiss Institute of Bioinformatics (SIB) at the Biozentrum, University of Basel, and Basel, Switzerland. In Silico—Molecular Interaction Analysis and Docking for Antimicrobial Peptides in MOS In silico molecular docking was performed to assess the docking ability of napin with Lipid II, 4PLB, and LipoXc. The 3D structure of napin, Lipid II, 4PLB, and LipoXc is downloaded from RCSB PDB. Proteins were prepared before docking and provided to Cluspro (v 2.0) [52]. The Cluspro web server is a widely used tool for protein–protein docking. It works on the fast Fourier transform correlation approach where simple scoring functions evaluate docking confirmation. Cluspro performs multi-stage protocol: rigidbody docking, energy-based filtering, evaluating structures based on clustering properties, and finally returning a small number of structures based on minimized energy. The server returns models based on energy and cluster size, among which one of the returned models was selected based on the lowest energy and size of the cluster. Binding energy surface properties are calculated, which in turn give the protein interface probability and the interaction site of two proteins. For surface properties calculation, Surface Racer 5.0 is used, which calculates the exact Accessible Surface Area (ASA), Molecular Surface Area (MSA), and cavities to the inner protein inaccessible to solvent from the outside. The output includes the surface area of the docked protein models for each residue in addition to those of individual atoms [53].

The top five ranked clusters are taken from Cluspro and run in the surface racer using van der Waal's radii as 2, and the radius for all the models was taken as 1.4 for surface area calculation. From the surface racer output, MSA was considered, and the best matching model is calculated. The difference between the MSA of the docked proteins model from the sum of the surface area of individual proteins and the larger surface area model is taken for further visualization of interactions. Furthermore, the interacting bonds within two docked protein complex molecular interactions within the docked protein complex were observed. Ligplot (v 2.2) [53] was used under an academic user license, and the DIMPLOT program of LigPlot + was executed to obtain the interactions across a selected protein–protein docked complex. The hydrophobic interactions and hydrogen bonds between the two docked proteins are represented in the figure where interacting residues are reported in Table 3. A computer-based molecular docking method was performed to identify the main compounds responsible for the antibacterial activity of ethanol extracts of different samples based on their possible binding mode and theoretical affinity toward bacterial topoisomerases (4PLB) and bacterial cell wall lipoprotein complex. The Cluster 2.0 web server web program for ligand–protein interaction is based on the fast Fourier transform correlation method that calculates docking validation by simple scoring functions [53, 54].

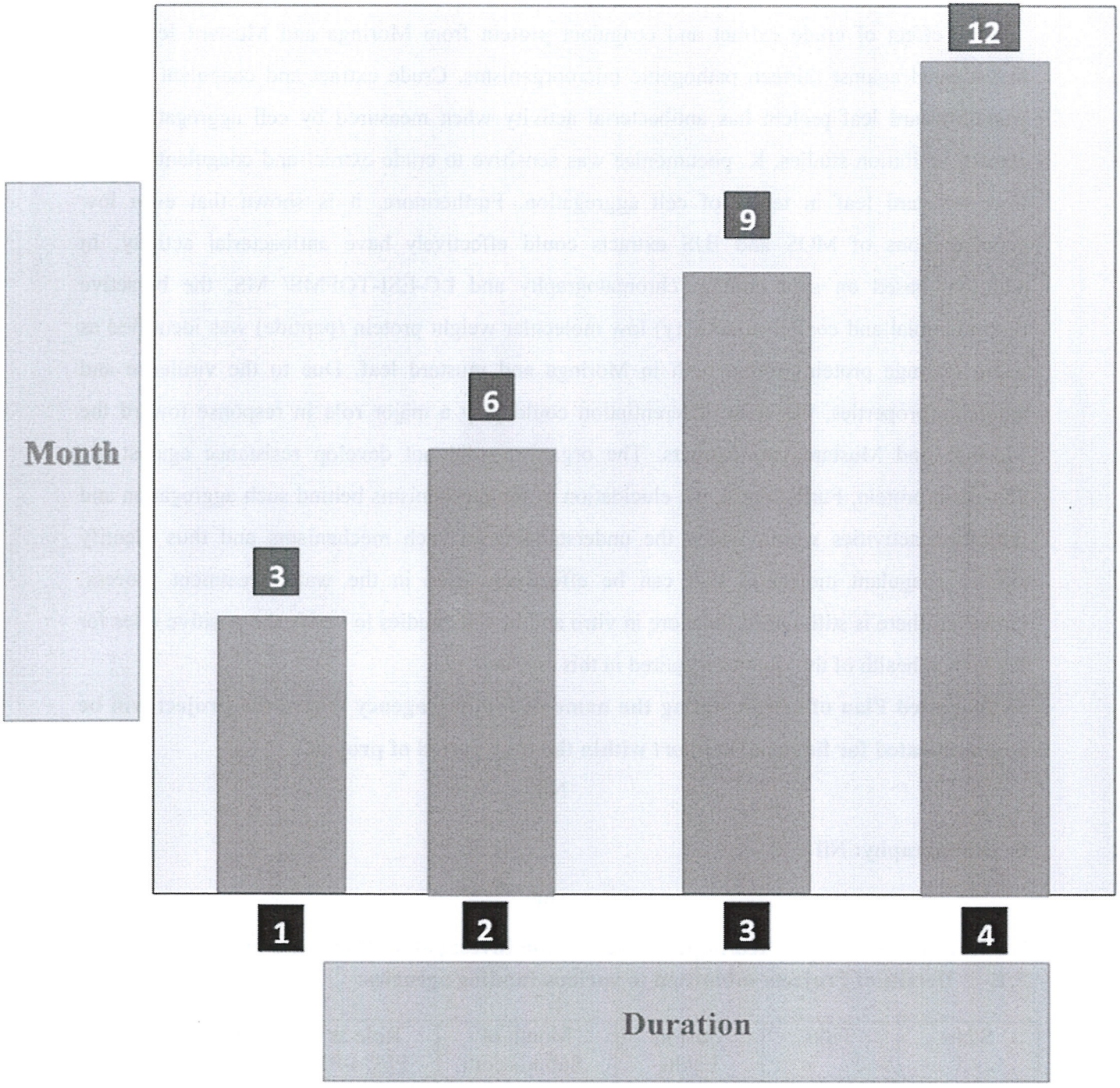
### 4.8. Antimicrobial Peptides (AMPs) with Limited Resistance

Our aim was to verify the inheritable nature of the evolved resistance from bacteria encountering AMPs frequently in their natural environments and evolving mechanisms to resist their action. Intrinsic resistance to AMPs can occur via passive or inducible mechanisms; we reconditioned the bacterial strain that showed good aggregation property, and one bacterial strain that did not show an aggregation property was selected to test the resistance development against

coagulant protein. E. coli cells were incubated with MOS, BJS, MCP, and BJP for 7 h at 37C. The cells were isolated from the culture and incubated again with protein for a growth inhibition test. This cycle was repeated 3 times to check the possibility of bacterial resistance development against coagulant protein. The limits of tolerance were confirmed as established in previous assays.

**4.2 Time Schedule of activities giving milestones through BAR diagram. (Maximum of 1/2 pages)**

| **S. No** | **Activity/ mile stolen** | **1st Year** | | | |
|---|---|---|---|---|---|
| 1 | Literature review | **1-3 month** | | | |
| 2 | Analysis of existing work | - | **4-6 month** | | |
| 3 | Designing & work initiated | - | - | **7-9 month** | |
| 4 | Statistics & Discussion with results | - | - | - | **10-12 month** |


Month
3
6
9
12
1
2
3
4
Duration

**4.3 Expected outcome within the time period of See Money Scheme**

The effect of crude extract and coagulant protein from Moringa and Mustard leaf were investigated against thirteen pathogenic microorganisms. Crude extract and coagulant protein from Mustard leaf protein has antibacterial activity when measured by cell aggregation and growth inhibition studies. K. pneumoniae was sensitive to crude extract and coagulant protein from Mustard leaf in terms of cell aggregation. Furthermore, it is shown that even low concentrations of MOS and BJS extracts could effectively have antibacterial activity. In addition, based on spin column chromatography and LC-ESI-TOFMS/ MS, the bioactive (antimicrobial and coagulant activity) low molecular weight protein (peptide) was identified as napin (storage protein) found both in Moringa and mustard leaf. Due to the virulence and antigenic properties, the stain differentiation could play a major role in response toward the Moringa and Mustard leaf extracts. The organisms did not develop resistance against the coagulant protein. Furthermore, the elucidation of the mechanisms behind such aggregation and inhibition activities would widen the understanding of such mechanisms and thus identify similar coagulant molecules that can be effectively used in the water treatment process. However, there is still a need for more in vitro and in vivo studies to verify the positive roles for the human health of the isolates acquired in this research.

**5. Suggested Plan of action stating the name of funding agency where the project will be communicated for financial support within the time period of project.**

Nil

**6. Bibliography:** Nil

Nil

**7. List of Projects submitted/implemented by the Investigators (Separate for Pi and Co-PI)**

**7.1 Details of Projects submitted to various funding agencies:**

| S.No | Title | Cost in Lakhs | Month of Submission | Role as PI/Co-PI | Agency | Status |
|---|---|---|---|---|---|---|
| 1 | NA | NA | NA | NA | NA | NA |

**7.2 Details of Projects under implementation**

| Sl. No. | Title | Cost in lakhs | Duration | Role as PI/ Co-PI | Agency |
|---|---|---|---|---|---|
| 1 | NA NA | NA | NA | NA | NA |

**7.3 Details of Projects completed during the last 5 years**

| Sl. No. | Title | Cost in lakhs | Duration | Role as PI/ Co-PI | Agency |
|---|---|---|---|---|---|
| 1 | NA | NA NA | NA | NA | NA |

**8. List of publications published by the Investigators, if any:**

**a) Principal Investigator**

| S. No | Author names | Title of paper | Name of Journal | Vol (Issue) | Page No. | Year |
|---|---|---|---|---|---|---|
| 1. | Deepika Velusami 1*, **Vijayakumar Raman 2*,** Muthu Gopal 3 | The Relationship between Non-Linear Analysis of Heart Rate Variability, QTc Interval and Cardiovascular Risk Factors in Young Individuals with Pre-Diabetes | Romanian Journal of Diabetes, Nutrition and Metabolic Diseases | 27(1) | 50-56 | 2020 |
| 2. | R. Yuvaraj1, **R. Vijayakumar1***, G. Bupesh2, S. Vasanth2 | Effect of Saraswatarishta on sleep deprivation induced behavioral changes in mice | Drug Invention Today | 12 (8) | 1741-1744 | 2019 |
| 3. | Velusami Deepika 1,2, **Raman Vijayakumar3** | Impact of Body Mass Index on Arterial Stiffness in Young Prehypertensives: A Cross Sectional Study | Journal of Research in Health Sciences | 18(1): e0040 2 | 1-6 | 2018 |
| 4. | Deepika.V1, **R.R.Vijaya Kumar2*,** S. Latha Vijaya Kumar3 And Dr.R.Srikumar4 | Impact of body mass index on corrected QT Interval in prehypertensives | International Journal of Pharma and Bio Sciences | 8(1): (B) | 266-270 | 2017 |
| 5. | S Latha, **R Vijaya Kumar,** BR Senthil Kumar, G Bupesh, TSV Kumar | Acute and repeated oral toxicity of antidiabetic polyherbal formulation flax seed, Fenugreek and Jamun seeds in Wistar albino rat | Journal of Diabetes & Metabolism | 7(3) | 1-7 | 2016 |
| 6. | **Vijaya kumar R,** Kishor kumar.C, | Prevalence of Prehypertension | Research Journal | 6(1) | 631-637 | 2015 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Christy A, Sasikala C | among school students in Puducherry | of Pharmaceutical, Biological and Chemical Sciences | | | |

**9. Budget**

| SI. No | Head | Amount (Rs.) |
|---|---|---|
| 1 | BP Apparatus, Stethoscopes, Body weight weighing machine, SPSS version 16 Chicago, IL, USA, ECG machine | 45000 |
| 2 | Consumables (gels bottles, cotton, sprit, testing charges, tools, etc.) | 10000 |
| 3 | Travel support for the purpose of research work. | 10000 |
| 4 | Contingency | 25000 |
| 5 | Others consumables | 10000 |
| | **Total** | **1,00,000** |

*In case of any joint proposal for purchasing a same equipment, each of the associated PLs is also required to give separate budget (without any clubbing) to avoid any ambiguity, if all the associated projects are not awarded by committee.

**10. Name of at least two subject experts from the Institute and one from the outside Institute with their contact details:**

| | |
|---|---|
| 1. **Dr. R. Ravindran,**<br>Professor in Physiology<br>IBMS, Chennai<br>**Mobile No**: 9444145990<br>**E-mail id:** ravindran89@gmail.com | 2. **Dr. Vasuki,**<br>Associate Professor in Physiology,<br>Priyadharshini Dental College, Thiruvallur<br>**Mobile No**: 9443793114<br>**E-mail id:** vasukiphysio@gmail.com |

## CERTIFICATE FROM THE INVESTIGATOR

**Project Title:** In Vitro and In Silico Screening and Characterization of Antimicrobial Napin Bioactive Protein in Brassica juncea and Moringa oleifera

It is certified that

1. I do hereby agree to submit a complete proposal for financial support to the external funding agency within the time period of SMS-2018.
2. I undertake that spare time on equipment procured in the project will be made available to other users.
3. I agree to submit a certificate from Institutional Biosafety Committee, if the project involves the utilization of genetically engineered organisms. I also declare that while conducting experiments, the Biosafety Guidelines of Department of Biotechnology, Department of Health Research, GOI would be followed in to.
4. I agree to submit ethical clearance certificate from the concerned ethical committee, if the project involved field trails/experiments/exchange of specimens, human & animal materials etc.
5. I agree to abide by the terms and conditions of SMS-2018, BIHER, and Chennai.

**Name and signature of**
**Principal Investigator**

**Name and signature of**
**Co-Principal Investigator**

**Date: 16.07.2019**
**Place: Pondicherry**

**Forwarded by Head of the Department**

**Signature of the Head**

DEAN
SRI LAKSHMI NARAYANA INSTITUTE OF MEDICAL SCIENCES
OSUDU, AGARAM VILLAGE,
KOODAPAKKAM POST,
PUDUCHERRY - 605 502

## PROJECT EVALUATION FORMAT

**Recommendation sheet**

| | |
|---|---|
| Name of the Principal Investigator | Dr.R.Vijayakumar |
| Name of the Co-Principal Investigator | Dr. S. Latha |
| Name of the Department | Physiology |
| Title of project | In Vitro and In Silico Screening and Characterization of Antimicrobial Napin Bioactive Protein in Brassica juncea and Moringa oleifera |
| Recommendation of the evaluation committee (Recommended/Revision/Not Recommended) | Recommended |
| Financial allocation recommended | Rs. 1,00,000/- |

| SI. No. | Head | Amount |
|---|---|---|
| 1 | BP Apparatus, Stethoscopes, Body weight weighing machine, SPSS version 16 Chicago, IL, USA, ECG machine | 45000 |
| 2 | Consumables- Gel bottles, cotton, sprit, testing charges, tools, etc. | 10000 |
| 3 | Travel support for the purpose of research work. | 10000 |
| 4 | Contingency | 25000 |
| 5 | Others consumables | 10000 |
| | **Total** | **1,00,000** |

Name and Signature of the Research Advisory Committee members with date.

Internal Quality Assurance Cell IQAC Pondicherry-02 SLIMS

(Dr. G. Jayalakshmi)