Sapthagiri College of Engineering

(Affiliated to Visvesvaraya Technological University, Belagavi& Approved by AICTE, New Delhi)
#14/5, Chikkasandra, Hesaraghatta Main Road, Bengaluru – 560057
Phone:080-28372800/1/2 www.sapthagiri.edu.in Fax: 080-28372797

Date: 04/09/2018

To,

The Principal,

Sapthagiri College of Engineering

Bangalore-560057

Sub- Sanction of Research grant for the research project "Gene Expression Study On Anti-Oxidant Enzyme Sod And Gpx"

In pursuance of the proceedings of the research committee and approval of the Principal through his letter dated 28/08/2018,the management in its meeting has decided to sanction the research grant. The management is pleased to sanction Rs.47,633/- **(Rupees forty seven thousand six hundred thirty three only)** for the Biotechnology department for carrying out the project, "Gene Expression Study On Anti-Oxidant Enzyme Sod And Gpx", by Prof. Shobha and Prof. Soumya.C as per the recommendation of RDECI.

The Principal is directed to facilitate the sanction of the amount and follow all the necessary procedure of the accounts and submit the utilization certificate after securing the same from the researcher.

Executive Director

Sri G.D. MANOJ
Executive Director
Sapthagiri College of Engineering
BENGALURU - 560 057.

Principal
Sapthagiri College of Engineering
Chikkasandra, Hesaraghatta Road,
Bangalore-560 057

Sapthagiri College of Engineering

(Affiliated to Visvesvaraya Technological University, Belagavi& Approved by AICTE, New Delhi)
#14/5, Chikkasandra, Hesaraghatta Main Road, Bengaluru – 560057
Phone:080-28372800/1/2 www.sapthagiri.edu.in Fax: 080-28372797

Date: 28/08/2018

To,

The Executive Director,

Sapthagiri College of Engineering

Bangalore-57

From,

The Principal,

Sapthagiri College of Engineering

Bangalore-57

Sub- Requisition for research grant for the research project.

With reference to the above subject, I hereby inform you that RDECI has approved the research project "Gene Expression Study On Anti-Oxidant Enzyme Sod And Gpx"submitted byby Prof. Shobha and Prof. Soumya.C of Biotechnology department after scrutinizing the research proposal.

Therefore, it is requested that an amount of Rs.47,633 (**Rupees forty seven thousand six hundred thirty three only**) may please be sanctioned for carrying out the above said research project.

Thanking you,

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Principal
Sapthagiri College of Engineering
Hesaraghatta Main Road

Sapthagiri College of Engineering

(Affiliated to Visvesvaraya Technological University, Belagavi & Approved by AICTE, New Delhi)
#14/5, Chikkasandra, Hesaraghatta Main Road, Bengaluru – 560057
Phone:080-28372800/1/2 www.sapthagiri.edu in Fax: 080-28372797

Date: 05/09/2018

To,

The Convener,

R&D, Entrepreneurship Committee & Incubation Center (RDECI),

Sapthagiri College of Engineering

Bangalore-560057

Sub- Sanction of Research grant for the research project "Gene Expression Study On Anti-Oxidant Enzyme Sod And Gpx "

The management of Sapthagiri college of engineering has sanctioned the Research grant of **Rs.47,633/- (Rupees forty seven thousand six hundred thirty three only)** for the research project "Gene Expression Study On Anti-Oxidant Enzyme Sod And Gpx "to be carried out by the department of Biotechnology.

Principal
Principal
Sapthagiri College of Engineering

Copy To,

All Departments

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Date: 23/08/2018

To,

The Principal,
Sapthagiri Collegeof Engineering,
Bangalore-560057,

Through HOD & RDECI

From

Shobha G and Soumya C
Departmentof Biotechnology
Sapthagiri Collegeof Engineering
Bangalore-560057

Respected Sir,

Subject: Requisition for the financial assistance for the project

With respect to above subject, I request your kind self to provide finical assistance to carry out the project entitled "**Gene expression study Antioxidant Enzyme SOD and GPX**". The research proposal and budget split up has enclosed along with this letter. Kindly consider the requisition and do the need full.

Thanking you

Your's sincerely

SAPTHAGIRI COLLEGE OF ENGINEERING
Dept of Bio-Tech
BANAGLORE-57

RDECI :

Head of the Department
Dept. of Bio -Technology
Sapthagiri College of Engineering
No. 57/1, Chikkasandra
Hesaraghatta Main Road
Bangalore -57

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

RESEARCH PROPOSAL

A. GENERAL INFORMATION

1	**About the project**		
a	Title of the project	:	**Gene expression study Antioxidant Enzyme SOD and GPX**
b	Subject area as per instruction	:	Biological & Agricultural Sciences
2	**Details of Principal Investigator**		
a	Name	:	Mrs Shobha G And Mrs Soumya C
b	Qualification	:	M.Sc, M.Phil, (Ph.D)
c	Designation	:	Assistant Professor
d	Department	:	Biotechnology
e	Years of teaching/research experience	:	10 & 14
f	Email ID	:	shobhag@sapthagiri.edu.in/ soumyac@sapthagiri.edu.in
g	Cell Number	:	9964591024/9538819506
h	**Details of the Head of the Department**		
i	Name of the Head of the Department		Dr. Rohit KC
j	Email ID	:	hodbt@sapthagiri.edu.in
k	Cell Number	:	9900833873

Signature of the Investigator.

SAPTHAGIRI COLLEGE OF ENGINEERING
Dept of Bio-Tech
BANAGLORE-57

Signature of Head of the Department

Head of the Department
Dept. of Bio-Technology
Sapthagiri College of Engineering
No. 57/1, Chikkasandra
Hesaraghatta Main Road
Bangalore-57

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

A. DETAILS OF THE PROJECT PROPOSAL

1.	**Title of the Project Proposal:**
	Gene expression study Antioxidant Enzyme SOD and GPX
2.	**Objectives of the proposal:**
	• DNA quantification • mRNA isolation • Genotoxicity assay of SOD and GPX
3.	**Background of the project:**
	Nanotechnology has transformed many frontages of the current society with its widespread applications in field of agriculture. Nanoparticles, in view of their intrinsic physico-chemical traits, are among likely contenders for modulating redox status there by altering the performance, quality and development of plant. Interactions amongst ecological influences and seed inner mechanisms control the capacity of a plant to germinate effectively. Hence germination is critical for deciding the plant density at the end, whenever planted, seeds should be able to germinate entirely and vigorously. Tomato (*Lycopersicon esculentum*) is a common vegetable crop of economic significance further, it is recognized as a classical plant as it contains a variety of secondary metabolites that can encourage biological, chemical and physiological examinations in expansion to an elective typical plant *Arabidopsis thaliana*. Tomato plant likewise is a perfect plant for numerous studies as it can proliferate in laboratories with least structure as compared to what is used for *A. thaliana*. The examination of antioxidants enzymes and its gene expression often tells about biotic and abiotic stress that occurs in the plant. The increased and decreased level of antioxidant enzymes tell about the development of reactive oxygen species (ROS) which tells about the DNA damage in plant. **Thus, the goal was to gain a better understanding regarding the Antioxidant Enzyme and of Tomato Plant against copper nanoparticle.Ultimately to develop recommended exposure limits for the plant development.**
4.	**Methodology :**

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

DNA extraction and Laddering

For DNA isolation, fresh leaf (100mg) was collected from treated and untreated control tomato plant and preserved at 20^0C for further study. The leaf was macerated in 500µL of extraction buffer (100mM Tris-HCl buffer pH 8.0, 25mM EDTA, 2M NaCl, 3% CTAB, 3% PVP, RNase) and proteinase K (20µl) was added to the plant extract. The samples were kept at 65°C for 30min with occasional mixing for every 10min. After the period of incubation, samples were centrifuged for 5 min at 10,000rpm at room temperature and pellet was discarded. An equal quantity of isoamyl alcohol-chloroform (1:24 v/v) and supernatant were taken and incubated overnight at -4°C followed by centrifugation at 12,000rpm for 10min. To the supernatant 1ml of 3M NaCl and 150µl silica matrix (1mg/ml) was added. The mixture was thoroughly vortexed and incubated for 5min at room temperature followed by centrifugation at 10,000rpm for 2 minutes at 4°C. The pellet is then washed twice using washing buffer (50% ethanol, 10mM Tris-HCl, 100mM NaCl, 1mM EDTA, pH 7.5) of 500µl and centrifuged at 10,000rpm for 2min. Under a sterile laminar hood, DNA was dissolved in 50µl of TE buffer (10mM Tris buffer pH 8, 1mM EDTA) and centrifuged for 10,000 rpm for 10min, supernatant was retained and stored at 4^0C until used. The DNA purity was measured by recording the optical density at 260nm and 280nm and integrity of DNA was observed by resolving on agarose gel (2.5%) in 1X TAE (Tris acetate EDTA) buffer.

Huanca-Mamani et al [275] protocol was followed for size-fractionalization of silica particles. The silicon dioxide was allowed to settle overnight after blending in 50ml of dH_2O. After discarding the supernatant, the pellet was re-suspended in 50ml dH_2O and allowed to settle overnight. After two-three repetition, the silica was used for DNA extraction.

2.2 Expression pattern of genes for SOD and GPX to Cu_2O NPs treatment

Total RNA extraction and cDNA preparation

Procedure: Total RNA were isolated from fresh leaf (100 mg) of treated and untreated control tomato

	plant with the total RNA isolation Mini Kit-Plant. Residual DNA was removed from the RNA samples with DNase-I. The quantity and quality of RNA was assessed with a Nano view spectrometer at 260nm and 280nm, whose ratio provides an estimate of RNA purity. RNA quality was confirmed by gel electrophoresis, containing 1.8% agarose and formaldehyde. The RNA is reverse transcribed into cDNA using M-MuLV RT-PCR Kit with Oligo (dT) according to the manufacturer's guidelines. The transcription levels of genes were analysed using qRT-PCR and the PCR System (Eppendorf). The PCR reaction mixture of a final volume of 25 µL consisting of 12.5µl of SYBR green master mix, 1µl of cDNA, dH_2O, and 1µl of each primer. The reaction mixtures in the beginning denatured for 10 min at 95°C and exposed to 40 cycles of 95°C for 15s and 58°C for 45s and 72°C for 30sec. The relative expression level was determined using the ΔΔCt method. It is then normalized to the Ct data relative to the transcript level of the ACTIN gene as an internal control.Electrophoresis was later performed in tris-EDTA buffer for 30min at 100 volts and 3µL of EtBr in 120mlof TBA buffer is used to stain the gel. The bands stained in the gel were observed and documented using a gel documentation system.		
5.	**Milestones with time schedule & work plan: 6 Months**		
6.	**List of equipment required for Phase-II for Project Implementation**		Kits Rs.47,633 apprx
7.	**Relevance, importance & application of the project:**		
	Researchers are now working to bridge a gap between agriculture and nanotechnology, so that of agri-nanotechnology can revolutionize the sector with new tools for disease detection, targeted treatment, enhancing the ability of plants to absorb nutrients, fight diseases, delivering nutrients or pesticides as appropriate, improve our understanding of the biology of different crops and thus potentially enhance yields. **At a same time nanoparticles and their increased use have raised concerns about their possible harmful effects within the environment.**		
10.	**Novelty/Uniqueness of the project proposal**	:	Over time, the results explains, nanomaterial in the agricultural inputs which may shows the positive and negative impact on growth of *Lycopersicon esculentum* and may contribute to the controversial

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

			debate on plant toxicity of nanoparticles.

Name & Signature of the Principal Investigator (with seal)

Name & Signature of Head of the Department (with seal)

Head of the Department
Dept. of Bio-Technology
Sapthagiri College of Engineering
No. 57/1, Chikkasandra
Hesaraghatta Main Road
Bangalore -57 .

SAPTHAGIRI COLLEGE OF ENGINEERING
Dept of Bio-Tech
BANAGLORE -57

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Date : 06/09/2018

From,

Research and Development cell,
Sapthagiri College of Engineering,
Bangalore-560057.

Through: HOD

To,

Principal investigator,
Sapthagiri College of Engineering,
Bangalore-560057.

Subject: Sanction of research grants Reg.,

The committee hereby informed that following projects have been approved for the academic year. The report and the outcome of the project as to be submitted to the committee after the completion of the project. The utilization certificate shall be given along with the final report.

Sl. No	Principal Investigator	Department	Project entitled	Amount Sanctioned
1	MrsShobha G/ MrsSoumya C	BT	**Gene expression study Antioxidant Enzyme SOD and GPX**	50,000/-

Convener

Copy To,
Principal
All Departments
IQSC

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Date: 20/10/2018

To,

Principal,
Sapthagiri College of Engineering
Bangalore-560057

Through HOD

From,

Shobha G,
Department of Biotechnology,
Sapthagiri Collegeof Engineering
Bangalore-560057

Respected Sir,

Sub: Procurement of Teaching Kits Reg.,

With reference to the letter received from R& D committee regarding approval of research project, we are here by requesting the procurement of teaching kits (RNA isolation kit for plant, qPCR Master mix, RT PCR Kit) which cost around **Rs 50, 000/-** (Fifty thousand) for the research project entitled "**Gene expression study Antioxidant Enzyme SOD and GPX**" to carry out. This will enhance the research potential to contribute for the improvement in the field of biotechnology.

Thanking you,

Yours Faithfully

SAPTHAGIRI COLLEGE OF ENGINEERING
BANGALORE-57

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bangaluru - 560 057

Head of the Department
Dept. of Bio -Technology
Sapthagiri College of Engineering
No. 57/1, Chikkasandra
Hesaraghatta Main Road
Bangalore -57

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bangaluru - 560 057

Date : 15/04/2019

To,

Research and Development cell,
Sapthagiri Collegeof Engineering
Bangalore-560057

Through HOD

From

Shobha G,
Departmentof Biotechnology
Sapthagiri Collegeof Engineering
Bangalore-560057

Sub: Submission of report and utilization details

With reference above cited subjected I am hereby enclosing project report entitled **"Gene expression study Antioxidant Enzyme SOD and GPX".** The item number (10 - RT PCR Kit, 11 - qPCR Master mix, 12-, RNA isolation kit for plant) mentioned in the invoice **SE/1819/565 Dated 23/10/2018** were utilized for research work. This for your kind information, please.

Sl No	Particulars	Quantity	Amount	
1	RT PCR KIT	2	12813/-	
2	QPCR MASTER MIX	2	17523/-	
3	RNA ISOLATION KIT FOR PLANT	2	17297/-	
TOTAL AMOUNT			47633/-	

Thanking You,

Copy,

Principal

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Head of the Department
Dept. of Bio-Technology
Sapthagiri College of Engg.
No. 57/1, Chikkasandra
Hesaraghatta Main Road
Bangalore-57

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

2018-0

Sakhala Enterprises

#966, "Shreeyaa" II Phase, V Stage, BEML layout, R.R. Nagar, Bengaluru-560098.
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No. : SE/1819/565
Date: 23.10.2018

To
The Principal
Sapthagiri College of Engineering
14/5, Chikkasandra
Hesaraghatta Main Road
Bangalore - 560057

PO No.: SCE/ADM/357/18-19
Date: 15th Oct 2018

Sl.No.	Product Description	Pack	Price	GST	Disc.	Amount	Qty	Sub Total	SGST	CGST	Total
1	DOT ELISA	15pr*	2,900	12%	1,015	1,885	6	11,310	679	679	12,667
2	ROCKET IEP	5pr*	4,300	12%	1,505	2,795	2	5,590	335	335	6,261
3	RADIAL IMMUNODIFFUSION	20pr*	3,600	12%	1,260	2,340	1	2,340	140	140	2,621
4	OUCHTERLONY DOUBLE DIFFUSION	10pr*	2,800	12%	980	1,820	1	1,820	109	109	2,038
5	BLOOD GROUP AGGLUTINATION	100pr*	800	12%	280	520	3	1,560	94	94	1,747
6	WIDAL TEST	20pr*	800	12%	280	520	3	1,560	94	94	1,747
7	COUNTER CURRENT IEP	10pr*	2,700	12%	945	1,755	2	3,510	211	211	3,931
8	RNA ISOLATION KIT	10pr*	4,900	12%	1,715	3,185	3	9,555	573	573	10,702
9	WESTERN BLOT	5pr*	7,600	12%	2,660	4,940	1	4,940	296	296	5,533
10	RT PCR KIT	5pr*	8,800	12%	3,080	5,720	2	11,440	686	686	12,813
11	qPCR MASTER MIX	20pr*	12,035	12%	4,212	7,823	2	15,646	939	939	17,523
12	RNA ISOLATION KIT FOR PLANT	20pr*	11,880	12%	4,158	7,722	2	15,444	927	927	17,297

Taxable Value	Central Tax Rate(%)	Central Tax Amount	State Tax Rate(%)	State Tax Amount	Total Tax Amount
-	9%	-	9%	-	-
84,715	6%	5,083	6%	5,083	10,166
-	2.5%	-	2.5%	-	-

Grand Total 94,880

Rs. Ninty Four Thousand Eight Hundred and Eighty Only.

HSN/SAC : 38220090

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SAKHALA ENTERPRISES B'lore-98

Authorised Signatory

Order once placed will not be cancelled or materials once sold will not be taken back.
Subject to Bengaluru Jurisdiction

Sapthagiri College of Engineering
SECURITY IN CHECKED
Date: 23/10/18
Time: 11:00
Sec. Sign: KAOY AE 6928

Received in good condition
23/10/18

23/10/18

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bangaluru - 560 057

Gene expression study Antioxidant Enzyme SOD and GPX

Submitted By

Mrs. ShobhaG and MrsSoumya C

Assistant Professor

Department of Biotechnology

To

DEPARTMENT OF BIOTECHNOLOGY

SAPTHAGIRI COLLEGE OF ENGINEERING

BENGALURU-560057

KARNATAKA, INDIA

2018

1. INTRODUCTION

Nanotechnology has transformed many frontages of the current society with its widespread applications in field of agriculture. Nanoparticles, in view of their intrinsic physico-chemical traits, are among likely contenders for modulating redox status there by altering the performance, quality and development of plant. Interactions amongst ecological influences and seed inner mechanisms control the capacity of a plant to germinate effectively. Hence germination is critical for deciding the plant density at the end, whenever planted, seeds should be able to germinate entirely and vigorously.

Tomato (*Lycopersicon esculentum*) is a common vegetable crop of economic significance further, it is recognized as a classical plant as it contains a variety of secondary metabolites that can encourage biological, chemical and physiological examinations in expansion to an elective typical plant *Arabidopsis thaliana*. Tomato plant likewise is a perfect plant for numerous studies as it can proliferate in laboratories with least structure as compared to what is used for *A. thaliana*. The examination of antioxidants enzymes and its gene expression often tells about biotic and abiotic stress that occurs in the plant. The increased and decreased level of antioxidant enzymes tell about the development of reactive oxygen species (ROS) which tells about the DNA damage in plant.

2. Materials and Methodology

2.1 DNA extraction and Laddering

Principle: The silica binding procedure and CTAB-PVP method are collectively used in DNA extraction protocol. During extraction, CTAB separates polysaccharides from DNA. Polyphenols forms hydrogen bonds with the PVP and precipitates in cell debris upon lysis of cell. In the existence of chaotropic salt silica helps in binding DNA. The RNase and proteinase enzymes present in the lysis buffer will eliminate the RNA and protein from samples through selective precipitation.

Procedure: For DNA isolation, fresh leaf (100mg) was collected from treated and untreated control tomato plant and preserved at 20^0C for further study. The leaf was macerated in 500µL of extraction buffer (100mM Tris-HCl buffer pH 8.0, 25mM EDTA, 2M NaCl, 3% CTAB, 3% PVP, RNase) and proteinase K (20µl) was added to the plant extract. The samples were kept at 65°C for 30min with occasional mixing for every 10min. After the period of incubation, samples were centrifuged for 5 min at 10,000rpm at room

SRI SRINIVASA EDUCATIONAL & CHARITABLE TRUST (R)

SAPTHAGIRI COLLEGE OF ENGINEERING

(Affiliated to Visveswaraya Technological University, Belgaum & Approved by AICTE - New Delhi)

UTILIZATION CERTIFICATE

Sl No	Particulars	Quantity	Amount
1	RTPCR kit	2	12813
2	QPCR master mix	2	17523
3	RNA isolation kit	2	17297
		Total	**47633**

Certified that Sapthagiri college of Engineering has provided partial financial support of **RS 47633/-**(Forty seven thousand six hundred and thirty three only) towardsGene expression study of antioxidant enzyme SOD and GPX project conducted by Biotechnology Department of Sapthagiri college of Engineering

Certified that I have satisfied myself that condition on which the grant in aid sanctioned has been duly fulfilled and that I have excised the following check to see that the money was actually utilized for the purpose for which it was sanctioned. The items are mentioned in the invoice SE/1819/565 dated 23/10/2018.

Kinds of check exercised

1. Bills

MAS & CO. CHARTERED ACCOUNTANTS VERIFIED BANGALORE 28/10

Signature of the Principal with seal

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Signature of Auditor with seal

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057