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)
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Ref. No.SMS-2015-O-04 Date: 17.08.2016

TO

Mr. C. Naveen Kumar
Assoc. Professor/Microbiology,
BIHER.

BHARATH INSTITUTE OF HIGHER EDUCATION & RESEARCH (Declared as Deemed to be University U/S 3 of UGC Act 1956) 11 AUG 2016 OFFICE OF THE DEAN-RESEARCH Selaiyur, Chennai-600 073. INDIA

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-2015 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: Phytochemical Analysis and Antifungal Activity of Ganoderma lucidum

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

Approved on: 10.08.2016

Payment details:

Voucher No.25 **Dated: 25.08.2016**

With Regards

Dean-Research

Bharath University

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

CASH / PAYMENT VOUCHER

Date 25/08/2016

V.No. 25

Debit _____ Amount

Rs. 1,00,000/—

PAID TO Dr. C. Naveen Kumar

RUPEES One lakh only

TOWARDS Seed Money Scheme-2015

BHARATH UNIVERSITY CHENNAI 600 073. INDIA.

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

PROPOSAL SUBMISSION

1. **Details of Principal Investigator**

Name	: Dr. C. Naveen Kumar
Designation	: Associate Professor
Highest Qualifications	: Ph.D.
Department	: Microbiology
E-mail	: navin.mmb@gmail.com
Contact no	: 9047765601
Date of Joining	: 13.04.2013

2. **Details of Principal Investigator**

Name	: Dr. R. Sri Kumar
Designation	: Associate Professor
Highest Qualifications	: Ph.D.
Department	: Microbiology
E-mail	: rsrikumar_2003@yahoo.in
Contact No	: 9442500300
Date of Joining	: 02.01.2012

Technical details

1. Introduction:

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine1. The herbal medicines serve the health needs of about 80% of the World's population, especially for millions of people in the vast rural areas of developing countries; more than 65% of the global population uses medicinal plants as a primary health care modality2. Plants have been a major focus of investigations for novel biologically active compounds3. However, filamentous fungi have been the producers of some of the most powerful secondary metabolites which have been developed into therapeutic agents4. In view of this, the searches for new anti-microbial agents from medicinal plants are even more urgent in the countries like India where infectious diseases of fungal origin are not only widespread, but the causative agents are also developing an increasing resistance against many of the commonly used antibiotics5. There is a constant search for new antibiotics because the existing drugs have unwanted toxicity and their inappropriate and indiscriminate use have led to an increase in antibiotic-resistant strains6. Numerous investigations have proved that medicinal plants as well as microorganisms contain diverse classes of bioactive compounds such as Tannins, Alkaloids, Flavonoids, Terpenoids, Phenols, etc7. The main aim of this current study was to detect the various bioactive components present in G. lucidum and also determine the antifungal activity to prove its use as a safe and potent antifungal agent.

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

Kamaraj, C., Rahuman, A., Siva, C., Iyappan, M., and Kirthi, AV. 2012. Evaluation of antibacterial activity of selected medicinal plant extracts from south India against human pathogens. Asian Pacific Journal of Tropical Disease. 2(1): S296-S301.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine (Abraham and Thomas, 2012). Traditional medicines are used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system. The herbal medicines serve the health needs of about 80% of the World's population, especially for millions of people in the vast rural areas of developing countries; more than 65% of the global population uses medicinal plants as a primary health care modality (Kamaraj et al., 2012). In recent years, many possible sources of natural antibiotics have been in use for several infectious diseases, mostly bacterial and fungal. In view of this, the searches for new anti-microbial agents from medicinal plants are even more urgent in the countries like India where infectious diseases of bacterial origin are not only rampant, but the causative agents are

also developing an increasing resistance against many of the commonly used antibiotics (Kamaraj et al., 2012).

2.1. International Status:

Ganoderma lucidum is a worldwide medicinal mushroom that is either cultivated or grows naturally on the bark of trees. Kenyan Ganoderma lucidum mushroom is conk like or kidney - like in shape. It is wood textured with 5 – 20cm in diameter and has a shiny surface which is black brown. In nature, it grows on dead or dying trees. The Chinese G. lucidum is also known as "Lingzhi" is red - vanished, kidney - shaped mushroom. Lingzhi is soft, cork - like and flat and the pores on the underside may be white or brown. The two mushrooms are different in morphology and appearance since they grow in different ecological zones

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

Preparation of extracts

The fruiting bodies of G. lucidum were obtained from MKV Organics, Puducherry. The extraction method of with certain modifications was used8. The dried fruiting bodies were grinded to a fine powder using a domestic blender. For preparing the extracts, methanol and ethanol were used as solvents to obtain the pharmacologically active compounds from the mushroom9-11. For every 1 gram of powder, 50 ml of solvent was used and was subjected to extraction using a Soxhlet extraction apparatus. After the completion of extraction, the supernatant was filtered through Whatman #1 filter paper. All solvent extracted fractions were evaporated to dryness to obtain residues. The extracts were stored at 4°C in air tight containers for further investigations12.

Phytochemical screening

The different qualitative chemical tests can be performed for establishing a profile of given extract for its chemical composition. The extracts were then subjected to qualitative chemical tests for various phytoconstituents like Alkaloids, Flavonoids, Carbohydrates, Reducing sugars, Tannins and Phenolic compounds, Cardiac glycosides, Terpenoids, Anthraquinones, Saponins, Volatile oils and Steroids13-15.

Detection of alkaloids

a) Mayer's Test

To a few ml of extract, one drop of Mayer's reagents was added by the side of the test tube. A white creamy precipitate indicated the test as positive.

Preparation of Mayer's Reagent

Mercuric chloride (1.358g) was dissolved in 60 ml of water and KI (5.0 g) was dissolved in 10 ml of water. The two solutions were mixed and made up to 100 ml with water.

b) Wagner's Test

To a few ml of extract, few drops of Wagner s reagent were added by the side of the test tube. A reddish-brown precipitate confirmed the test as positive.

Preparation of Wagner's Reagent

Iodine (1.27 g) and KI (2 g) were dissolved in 5 ml of water and made up to 100 ml with distilled water.

Detection of Carbohydrates

a) Molisch's test

To 2 ml of extract, two drops of alcoholic solution of α-naphthol was added, the mixture was shaken well and 1 ml of conc. H_2SO_4 was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.

b) Benedict's Test

To 0.5 ml of extract, 1 ml of Benedict s reagent was added. The mixture was heated on a boiling water bath for 2 mins. A characteristic-colored precipitate indicated the presence of sugar.

Benedict's Reagent

Sodium citrate (173g) and Na_2CO_3 (100g) were dissolved in 800 ml of distilled water and boiled to make it clear. $CuSO_4$ (17.3g) dissolved in 100 ml distilled water was added to it.

Test for Glycosides

a) Legal's test

To the extract, few drops of 10% NaOH were added to make it alkaline. Sodium nitroprusside was added to the solution. Presence of blue coloration indicated the presence of glycosides in the extract.

b) Keller-Killiani test (for cardiac glycosides)

To 2 ml of extract, 2 ml glacial acetic acid is added, followed by one drop of 5% $FeCl_3$. Conc. H_2SO_4 is added from the side of the test tube. Reddish brown ring appears at the junction of the two liquid layers indicating the presence of cardiac glycosides.

Detection of Proteins and Amino Acids

a) Millon's Test

To 2 ml extract, few drops of Millon's reagent were added. A white precipitate indicated the presence of proteins.

b) Biuret Test

An aliquot of 2 ml of extract was heated with 1 drop of 2 % $CuSO_4$ solution. To this 1 ml of ethanol (95%) was added, followed by excess of KOH Pellets. Pink colour in the ethanolic layers indicated the presence of proteins.

Detection of Flavonoids

a) Shinoda test (Magnesium Hydrochloride reduction test)

To the test Solution, few fragments of Magnesium ribbon were added and concentrated Hcl was added drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

b) Alkaline reagent test

To the test solution few drops of sodium hydroxide solution was added; formation of an intense yellow color, which turned to Colourless on addition of few drops of dil. acid, indicated the presence of flavonoids.

Detection of Phytosterols

a) Libermann Burchard's Test

To the extract, 3 ml of acetic anhydride was added and mixed. To this one drop of concentrated H2SO4 were added slowly along the sides of the test tube. An array of colour change showed the presence of phytosterols.

Test for Triterpenoids and Steroids

a) Libermann Burchard's Test

Extract was treated with few drops of acetic anhydride, boiled and cooled. Conc. H2SO4 was added from the sides of the test tube, showed a brown ring at the junction of two layers and the upper layer turning green showed the presence of Steroids and formation of deep red colour indicated the presence of triterpenoids.

b) Salkowski test

Extract was treated with few drops of conc. H2SO4, shaken well and allowed to stand for some time; red color at the lower layer indicated the presence of Steroids and formation of yellow colored lower layer indicated the presence of Triterpenoids.

Detection of Phenolic Compounds and Tannins

a) Ferric Chloride Test

To the extract, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

b) Lead Acetate Test

To the extract, 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Fungal Test strains used

A total of five fungal species were tested. Candida albicans (ATCC-10231), Aspergillus niger (ATCC-6275), Aspergillus flavus (ATCC-204304), Aspergillus fumigatus (ATCC-16907) and Cryptococcus neoformans (ATCC-208821).

Culture media and inoculum preparation

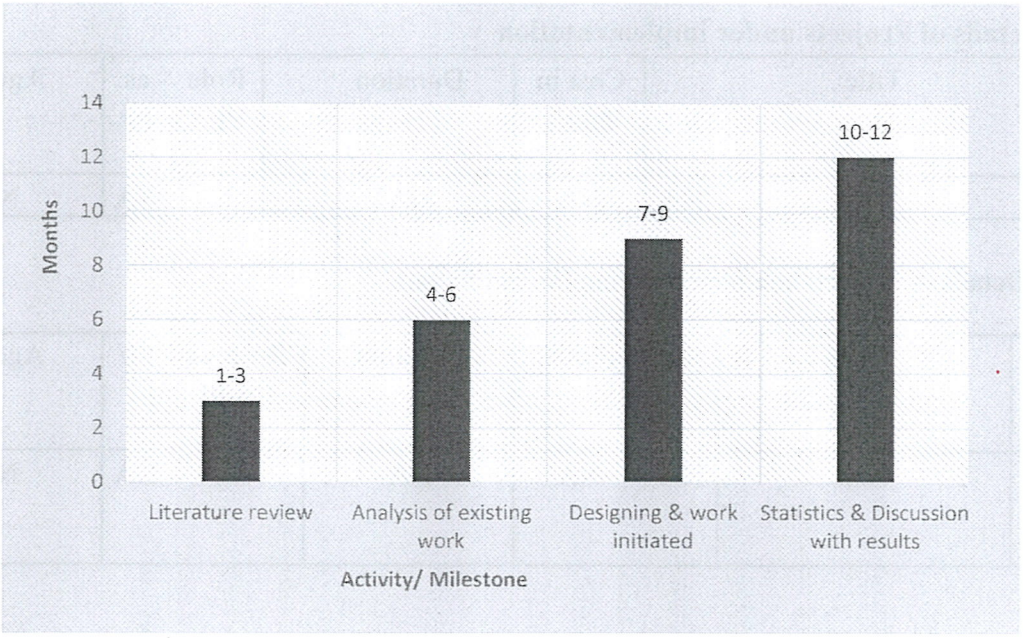
The isolates were grown on Sabouraud dextrose agar (Himedia) for 48 h at 35°C. The inoculum preparation followed the directions of document M27-A of the NCCLS16. Thus, the optical density (OD) of a 0.5 McFarland standard at 530 nm was measured five times on different days. Therefore, a suspension of each of the yeasts in sterile distilled water was adjusted in Bausch & Lomb spectrophotometer to that OD530 range.

Minimum Inhibitory Concentration (MIC)

The MIC was determined as the lowest concentration of the extract which inhibited the growth of the tested fungi. A broth micro-dilution bioassay in 96-well micro titer polystyrene plates was used to determine MIC. The method of was followed with modifications17. The wells of each column (1-12) were filled with 50 µl of sterilized RPMI broth (except the first well of each column). 100 µl of the extracts (methanol and ethanol) having a concentration of 10 µg/ml was added to the first well of columns 4-12. Serial two-fold dilutions were made of the 10 µg/ml extract with the broth in the 07 consecutive wells of the columns. The concentration of the extracts ranged from 10 to 0.0625 µg/ml. Next, 50 µl of the fungal inoculam were added to each well so that the final volume of each well was 150 µl. The first & second column of the plate served as the positive and negative control. The plates were covered and then incubated at 37°C for 24h. After 24-48 hrs, 40 µl of 0.2 mg/ml iodonitrotetrazolium chloride was added to each well and the plates were further incubated at 37°C for 30 min. Fungal growth in the wells was indicated by development of red-pink color, while growth inhibition was indicated by no change in the colour of cell suspensions. The MIC of each extract is defined as the lowest concentration inhibiting the growth of the fungi and was recorded.

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**



4.3 Expected outcome within the time period of See Money Scheme

The results obtained from this work showed that extracts of *G. lucidum* medicinal mushroom screened exhibit antifungal effects present more in methanolic extract than ethanol extracts against the fungal strains. This study also supports the traditional usage of the studied plants and suggests that *G. lucidum* extracts possess compounds with antifungal properties that can be used as antifungal agents in new drugs for the therapy of infectious diseases caused by pathogens.

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.	**Naveen Kumar C,** Sri Kumar R, Swathi R, Prabhakar Reddy E, Chidambaram R.	Role of Ganoderma lucidum against trizole drugs resistant Aspergillus species	International Journal of Research Pharmaceutical Sciences	9(4)	1189-1195	2018
2.	S Ayyappan, Sachu Philip, N Bharathy, V Ramesh, **C Naveen Kumar,** S Swathi, A Arun Kumar	Antioxidant status in neonatal jaundice before and after phototherapy	Journal of pharmacy & Bioallied sciences	7(1)	S16-19	2015
3.	KP Shiva Govindan, Saleem Basha, V Ramesh, **C Naveen Kumar,** S Swathi	A comparative study on serum lipoprotein (a) and lipid profile between rheumatoid arthritis patients and normal subjects	Journal of Pharmacy & Bioallied Sciences	7(1)	S22-25	2015
4.	Jayaranjani.K Jayarani.K, Sandhyarani.T, **Naveen Kumar. C,** Swathi.S	Detection of MBL Producing Pseudomonas aeruginosa in Tertiary Care Hospital, Pondicherry	International Journal of Recent Scientific Research	5(8)	1460-1463	2014
5.	Janani.S Sandhyarani T, Jayarani.K, Sai Ravikiran B, **Naveenkumar. C**	Microbiological Profile and Spectrum of Drug Susceptability In Asymptomatic Bacteriuria Among Antenatal Women	Universal Research Journal Of Medical Sciences	1(1)	13-16	2014

b). Co-Principal Investigator

S. No	Author names	Title of paper	Name of Journal	Vol (Issue)	Page No.	Year
1.	S Latha, R Venkataramanan, **R Srikumar**, RV Kumar	Effect of Triphala on noise stress induced alteration in glucocorticoid and carbohydrate metabolism.	International Journal of Pharma and Bio Sciences	6(2)	1-15	2015
2.	Manikandan Sundaramahalingam, **Srikumar Ramasundaram**, Sheela Devi Rathinasamy, Ruvanthika Pulipakkam Natarajan, Thangam Somasundaram	Role of Acorus calamus and alpha-asarone on hippocampal dependent memory in noise stress exposed rats.	Pakistan journal of biological sciences: PJBS	16(16)	770-778	2013

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	Other's 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. Florida,** Research Scientist, Sree Balaji Medical College and Hospital, Chennai **Mobile No**: 9940027169 **E-mail id:** biozonediagnostic@gmail.com	2. **Dr. Suba,** Professor in Microbiology, Rela Transplantation Institute, Chennai **Mobile No**: 9962526457 **E-mail id:** subamicro@gmail.com

CERTIFICATE FROM THE INVESTIGATOR

Project Title: Phytochemical Analysis and Antifungal Activity of *Ganoderma lucidum*

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-2015.
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-2015, BIHER, and Chennai.

Name and signature of
Principal Investigator

Name and signature of
Co-Principal Investigator

Date: 22.07.2016
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. C. Naveen Kumar
Name of the Co-Principal Investigator	Dr. R. Sri Kumar
Name of the Department	Microbiology
Title of project	Phytochemical Analysis and Antifungal Activity of *Ganoderma lucidum*
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. P. Jayakumar)