Project Proposal On

""Deciphering molecular mechanisms involved in criss-cross talk between sesquiterpene-triterpene formation in S. album""

Submitted to:

Department of Biotechnology
Ministry of Science and Technology
Government of India
New Delhi, India

Submitted By
Project Coordinator:

Dr. Arti Rani
(Vittal Mallya Scientific Research Foundation - Bangalore)

Part 1: General Information

1. **Name of the Institute/University/Organisation submitting the Project Proposal :**

   Vittal Mallya Scientific Research Foundation

2. **State:** Karnataka

3. **Status of the Institute:** Others

4. **Designation of the Executive Authority of the Institute/University forwarding the application :**

   Director

5. **Project Title :**

   "Deciphering molecular mechanisms involved in criss-cross talk between sesquiterpene-triterpene formation in S. album"

6. **Category of the Project :** R & D

7. **Specific Area :** Biosystems & Bioprocess Engineering

   Is the Proposal Submitted Under Specific Call for Proposal: No

8. **Project Duration :** 3 Years and 0 Months

9. **Project Total Cost (Rs):** 5553000.00

10. **Single/Multiple-Institutional:** Single-Institute

11. **If the project is multi-institutional, please furnish the following :**

    Project Coordinator : N/A

    Affiliation : N/A

    Address : N/A

12. **Project Keywords:** santalol, squalene, cell suspensions, sandalwood

13. **Require Regulatory Clearance:** No

    Uploaded Regulatory Clearance Document: Not uploaded

14. **Require Ethical Clearance:** No

15. **Industry Collaboration:** No

16. **Project Summary (Not to exceed one page. Please use separate sheet).**

    S. album is a medicinal plant of great interest. The plant is a rich source of sesquiterpenes key constituent of which is santalol- a biological compound with anticancerous, anti-inflammatory, antipyretic and antiulcerogenic activities. The volatile oil containing santalol is accumulated in the heartwood of tree after maturation. Sandalwood is also a source of still another triterpene – squalene that accumulates in the pericarp and fruit of the tree. Squalene has emerged as a specialty chemical being important in nutraceutical, pharmaceutical, vaccine and cosmetic industries due to the anticancer, antioxidant, skin hydrating, immune stimulating and emollient activities. The main source of squalene is

shark liver oil, but alternate sources are being sought to secure sustainable supply of squalene and conserve the marine sources.
The present work will be carried out with the aim of generation of both squalene and santalol in the cell suspension cultures of sandalwood and study the mole

Part 2: Particulars of Investigators

Principal Investigator:

1) Name: Dr. Arti Rani

Date of Birth: 26/02/1978

Designation : PI

Department: Biotechnology

Institute/University: Vittal Mallya Scientific Research Foundation

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Number of Research projects being handled at present: 1

Co-Investigator:

1) Name: Dr. Anil Kush

Date of Birth: 13/09/1956

Designation : Director

Department: Biotechnology

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Number of Projects being submitted/pursued/carried out by PI(s) at present: 0

Part 3: Technical Details Of Project

Introduction:

17.1 Origin of the proposal

Santalum album commonly known as “Chandan” is a medicinal plant of India and is a member of family Santalaceae. The plant has gained importance due to scented heartwood accumulating essential oil. The essential oil is of great economic value due to the presence of pharmaceutical components called sesquiterpenes such α-santalene, β-santalene, epi-β-santalene, tran-α-bergamotene, β-bisabolene, β-curcumene, α-santalal, β-santalal, (E)-neralidol, α-bisabolol, β-bisabolol, dihydro-α-santalol, cis-α-santalol, (Z)-trans-α-bergamotol, trans-α-santalol, epi-β-santalol, cis-β-santalol, trans-β-santalol.

In addition to the above major components, sandalwood pericarp and seed oil is known to accumulate minute amounts of economically important triterpene called squalene (Zhang et al. 2012; Hettiarachchi et al. 2013). Squalene is a 30-carbon triterpene synthesized as a biochemical intermediate in plants, animals and fungi. It plays vital role in the biosynthesis of sterols such as cholesterol, steroid hormones and vitamin D in human body. In plants and fungi, it acts as the precursor of stigmasterol and ergosterol, respectively.

Both santalol and squalene are of great economic value. Santalol possesses anti-inflammatory, antipyretic, antiulcerogenic, antifungal, antiviral, antibacterial, antioxidant, antispasmodic (muscle relaxant), astringent, diuretic and anticancerous activities (Guo Shi-Kui et al., 1983; Shankaranaryana and Parathasarthi, 1985; Shankaranaryana and Kamala, 1989; Sivaramakrishnan and Shankaranarayana, 1990; Scartezzini and Speroni, 2000; Kaur et al. 2005; Szkopiñska and Plochocka, 2005; Arasada et al., 2008). Similarly many studies have proven the anticancer, antioxidant, drug carrier, detoxifier, skin hydrating, immune stimulating and emollient activities of squalene in animal models and in vitro environments indicating huge potential in nutraceutical and pharmaceutical industries (Huang et al. 2009; Kim and Karadeniz 2012). Squalene plays an essential role in protecting skin from free radical oxidative damage. It is used in treating skin disorders like seborrheic dermatitis, acne, psoriasis, or atopic dermatitis. In cancer therapy, squalene acts as a potentiating agent for anticancer drugs such as ACNU (3-[(4-amino-2-methyl-5-pyrimidinyl) methyl]-1-(2-chloroethyl)-1-nitrosourea) and bleomycin. Recently squalene is being utilized in nanomedicines due to unique, dynamically folded molecular conformation and biocompatible qualities. The technique described as squalenoylation technology involves linking a biologically active compound (anticancer, antibiotic, antiviral, MRI imaging agent) to squalene to form a drug bioconjugate called nanoparticles. Such nanoassemblies are proven to have high pharmacological activity with reduced toxicity (Couvreur 2016). In addition to its use in cosmetics and vaccine, squalene being a hydrocarbon can also be utilized as raw material to replace petroleum and as feedstock for chemical industry.

Owing to huge importance of both the chemicals and limited sources, generation of these compounds in significant amounts becomes imperative. Though production options such as total chemical synthesis, semisynthesis from the isolated precursors, overexpression of regulatory pathway genes in microbial systems are available, utilization of medicinal plant for synthesizing pharmaceutically important compound looks more promising. In this regard we selected sandalwood (Santalum album), a medicinal plant with active terpenoid pathway for generation of these compounds in cell suspensions. Use of cell suspension cultures looks attractive because the production of biomolecules occurs under sterile in vitro conditions with high level of containment and the system is simple with low cost of downstream processing

(Franconi et al. 2010; Kim et al. 2008). Fast production of desired biomolecules gives extra advantage because the cells lines can be generated in large scale in minimal time without waiting for specific time or season as in plants (Shaaltiel el al. 2007; Aviezer et al. 2009). Homogeneous and synchronous nature of cell suspension cultures makes them more amenable to large scale production of phytochemicals. Numerous reports are available underscoring the efficient production of secondary metabolites products in the form of pharmaceuticals, flavours, fragrances (Kolewe et al. 2008; Weathers et al. 2010; Wilson and Roberts 2012).

Getting squalene from the cell suspensions of vegetal origin will definitely be a preference in pharma and cosmetic industry as it will be an unlimited, non-destructive and pollution free source of the compound generated without any genetic transformation. In this project proposal our attempt will be to induce both santalol and squalene formation in cell suspension cultures of sandalwood and also to study the molecular mechanisms leading to the production of diverse compounds of immense importance.

17.2 Rationale of the study supported by cited literature (b) Hypothesis (c) Key questions.

A) Rationale of the study:

Through detailed study on sandalwood cell suspension cultures we found that the sandalwood cell lines responded differently in different conditions leading to diversion of pathway either towards cyclization of farnesyl pyrophosphate (FPP) or dimerization. Cyclization of FPP leads to the formation of cyclic sesquiterepnes such as santalene, bisabolenes and bergamotenes whereas dimerisation of FPP lead to the formation of triterpene called squalene. In sandalwood santalol is detected only in the heartwood and roots of the mature tree whereas squalene is detected only in the pericarp and seed oil. This could be due to the fact that in mature tree the cyclases are proactive and whole FPP available is used for sesquiterpene formation in the heartwood of the tree. However at cellular level where we can control the culture conditions, we can divert the pathway towards santalol formation by providing conditions leading to upregulation of santalene synthase and downregulation of squalene synthase or to the formation of squalene by upregulation of squalene synthase and downregulation of santalene synthase.

B) Hypothesis

Both sesquiterpenes and triterpenes biosynthesis follow a common biosynthetic pathway called terpenoid pathway. In plants the terpenes are synthesized by combined involvement of mevalonate pathway (MVA; in cytosol) and 2-C-methyl-D-erythritol-4-phosphate pathway (MEP; in plastids). In MVA pathway, sequential condensation of three acetyl-CoA units generates 3-hydroxy-3 methylglutaryl-coenzyme A (HMG-CoA), which in turn is converted into mevalonate in an irreversible reaction catalyzed by HMG-CoA reductase (HMGR). Six carbon mevalonate is sequentially phosphorylated and decarboxylated to give rise to isopentenyl pyrophosphate (5C; IPP). In MEP pathway, condensation of glyceraldehydes-3-phosphate and pyruvate leads to the formation of 1-deoxy-D-xylulose-5-phosphate (DXP), reductive isomerization of which gives rise to MEP involving 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) enzyme. Further MEP is transformed into IPP and dimethylallyl pyrophosphate (5C; DMAPP). Once isoprenoids are formed; both MVA and MEP pathways follow the same enzymatic route. One molecule of IPP condenses with one molecule of DMAPP to produce monoterpene called geranyl pyrophosphate (10C; GPP). One molecule of GPP condenses with one more molecule of IPP to produce hydrophobic sesquiterpene called farnesyl pyrophosphate (15C; FPP). Both the reactions are catalyzed by farnesyl diphophate synthase (FDS). Once FPP is formed, it acts as the precursor both for sesquiterpenes and triterpenes. Cyclization of FPP mediated by santalene synthase leads to the formation of sesquiterpenes whereas dimerization of FPP mediated by squalene synthase (SQS)

leads to the formation of squalene (Zhao et al. 2010). In the present project our attempt will be to generate santalol and squalene in the same cell lines but at different cultural conditions.
As depicted in the figure below, cyclization involving cyclases leads to the formation of most important components such as santalenes, bergamotene and bisabolene. Head to head condensation of FPP with FPP give rise to triterpenes such as squalene. Thus the sandalwood cell lines capable of producing FPP in abundance can be diverted towards sesquiterpenes by upregulation of cyclases and downregulation of squalene synthase (SQS). Similarly, same the cell lines can be diverted for the production of squalene by downregulation of cyclases and and upregulation of SQS.

C) Key Questions
1. What are the factors responsible for upregulation or downregulation of the genes in lower terpenoid pathway?
2. Does the regulation of SaSS and SQS at transcriptional level or translational level?

17.3 Current status of research and development in the subject (both international and national status)

International Status:
Though much work is done on sandalwood, there are no reports about the use of cell suspension cultures for production of terpenes. There is paper by Cravadore et al. (2012) where ACC mediated sesquiterpene formation is achieved in the calli.
17.2 National status:
India has a unique position in terms of Sandal cultivation area. Information is available on the cultivation practices in forest, micro-propagation and insect pest management. Somatic embryogenesis (Rao and Bapat, 1995; Rai and McComb, 2002) and regeneration of whole plants by embryogenesis from cell suspension cultures of sandalwood (Lakshmisita et al., 1979) was developed and is used for its propagation by forest department and wood research institute in Bangalore. Genetic diversity in S. album has been studied using isozyme variation (Suma and Balasundaram, 2003) and RAPDs (Shashidhara et al., 2003; Suma and Balasundaram, 2004).
In search of transgenic sandalwood, Veena and Rao (1998) standardized a protocol of Agrobacterium mediated transformation using cotyledon stage embryos. Lakshi Sita and Bhattacharya (1998) isolated and cloned a proline-rich protein from the leaves of S. album and inserted into the genome of sandalwood to study the potential of this gene in resistance against spike disease prevalent in sandalwood. In 2008, Shekhawat and co-workers (2008) reported a method of Agrobacterium mediated transformation using embryonic cell suspension cultures instead of embryos or hypocotyles. Cell suspension cultures of sandalwood grown in air-lift bioreactor and shake flask cultures are standardized by Misra and Dey (2013) capable of producing shikimic acid in 14 days as compared to 6 years of maturity. At molecular level an attempt has been done by Srivastva et al. (2015) where cloning and functional characterization of five genes encoding two sesquisabinene synthases and a bisabolene synthase in addition to santalene synthase and farnesyl diphosphate synthase is achieved using the Illumina next generation sequencing of S. album.
In VMSRF, our team has isolated two key regulatory genes (SaFDS and SaSS) from Santalum album and has studied the tissue specific expression pattern (Rani et al. 2013; DBT Ref no. BT/PR 11689/PBD/17/605/2008). We have generated highly proliferating cell lines from sandalwood embryos and transformed the cells with SaFDS and SaSS for increasing the santalene production [(Ref Number (BT/PR12111/TRM/120/12/2014)]. In continuity to this, in the present project proposal our attempt will be to induce santalol and squalene biosynthesis in cell suspension cultures without any genetic manipulation and study the different factors

responsible for diversion of branch point either towards sesquiterpene formation or triterpene formation.

17.4 The relevance of the proposed study

Though Sandalwood is widely used and has extreme economical value, little is known at molecular level of the plant. Enzymes and pathways that lead to synthesis of sesquiterpenes and triterpenes largely remain unexplored. A thorough knowledge of the molecular mechanism could help us generate leads in the in-vitro synthesis of both sesquiterpenes and triterpenes. This would help obligate the destructive method of deriving compounds thereby saving many trees. Achieving this if we are capable of both santalol and squalene production in the same cell lines, it will be a great value addition in biomedicine as both the compounds offer a bundle of economic value.

17.5 The Outcome of Proposed Study

1. An opportunity to build on and complement research that is ongoing in many laboratories throughout the world.
2. An approach to identify genes and study their regulation.
3. The compounds santalol and squalene can be produced for commercialization completely in sterile and stringent conditions in the same cell lines but in different media.

17.6 Preliminary work done so far

19. The Preliminary work done so far
19. A. Initial leads from previous DBT funded project:
With the financial support of DBT we have taken important leads that motivated us to continue the work in a more elaborative way. Previously, in search of novel genes of terpenoid pathway, a suppression subtraction hybridization library (SSH) was prepared using two contrasting mRNA samples isolated from wood of young (<2 years) and mature (>10 years) S. album trees and screening was done to identify the partial sequences of the genes. Limited screening led to isolation of SaFDS and SaSS, the genes of terpenoid pathway which were raised to full length by RACE. Both the genes were cloned in bacterial expression vector and functionality was checked after IPTG induction and Ni-NTA column purification. The product formation by in vitro assay was confirmed by GC analysis of the samples and so the ORF of both the genes was cloned downstream to constitutive promoters 35S and EntCUP in plant expression vector pCAMBIA. The embryos of the sandalwood seed were used for callus formation in tissue culture and the fragile callus was used to generate highly proliferating cell lines (Fig. 2). The cell lines were further used for Agrobacterium mediated transformation using gene construct containing SaFDS and SaSS under control of constitutive promoters. The untransformed control and transformed cell lines were analyzed for sesqui terpene formation. The details are as follows:

19A.1 Plant Materials and generation of sandalwood callus
Santalum album L. seeds were collected from Kunigal stud farm located in the town of Kunigal in the Indian state of Karnataka. The seeds were surface sterilized with 2.5% sodium hypochlorite for 30 min followed by 3-4 washes with sterile autoclaved water. The sterilized seeds were scalpel shelled in laminar hood, allowed to dry under air flow in laminar hood and cut into two halves with the help of scalpel. Half seeds (Fig. 2A) were inoculated in Woody Plant Medium (WPM) supplemented with thidiazuran. Petri plates containing half seeds were kept in plant tissue culture room with 16 h day and 8 h dark photoperiod (temperature, 25±1ºC; light intensity, 60-70 µE m-2 s-1) for 30 days till fragile callus (Fig. 2B) was formed.
19A.2 Generation of highly proliferating cell lines
Part of the fragile callus was inoculated in liquid WPM containing 2,4D (1.0 mg/L) and

incubated in the orbital shaker (New Brunswick Scientific, Innova®40) at 28°C with 90 rpm for three months. Subculture was done every fortnight in a ratio of 1:5 of old suspension and fresh medium. Coarse sandy cell suspension (Fig. 2C) was obtained after three months. Subsequent sub cultures of coarse sandy culture led to the formation of sandy culture (Fig. 2D) followed by fine milky culture (Fig. 2E).

19B. Initial leads for the proposed project:

19B.1 Optimization of WPM medium for sandalwood cell suspensions

WPM medium for sandalwood cell suspensions was optimized by monitoring the growth rate of cells for six weeks (42 days) at different sucrose concentrations, media strength, pH of the media and 2,4 D hormone concentrations. Initially, the cryopreserved highly proliferating cell lines were inoculated in 1XWPM medium (100 ml) containing 3% sucrose and 2,4 D (1.0 mg/L) and incubated in the orbital shaker at 28°C with 90 rpm for three days. To see the effect of sucrose on growth, revived culture (10 ml) was inoculated in 1XWPM (100 ml) medium containing different sucrose percentage (1, 2, 3, 4, 5 and 10%). Sucrose concentration of 50g/L (5%) was found to be best for cell growth and selected for optimizing rest of the parameters. To study the effect of medium strength, 10 ml of the revived cells were transferred to three different flasks containing 100 ml of ½XWPM, 1XWPM and 2XWPM medium and 5% sucrose. Best results in terms of cell growth were observed at 2XWPM and thus same was used for further study. To investigate the effect of pH on cell growth, 10 ml of the revived cells were transferred to four different flasks containing 2XWPM and 5% sucrose set to pH values 4.0, 6.0, 8.0 and 10.0. The pH of the medium was adjusted with 0.1 N NaOH prior to autoclaving. A pH of 6.0 was found to be optimal for cell growth and selected for further studies. To find the effect of 2,4D hormone on cell proliferation, 10 ml of the revived cells were transferred to three different flasks containing 100 ml of 2XWPM and 5% sucrose set to pH 6.0. The hormone 2,4D was added to final concentrations of 1, 1.5 and 2 mg/L and 2,4D concentration of 1.5 mg/L was found to optimum for growth of the cells (Fig. 3A-3D).

19B.2 Accumulation of santalol in the cells grown in shake flasks

Sandalwood cell suspensions were grown in optimized medium 1 and harvestation was done after 14 days of culture. The cells were centrifuged and extracted in methanol. GC analysis of the extract showed the presence of sesquiterepnes such as santalol and bergamotol. In addition, isogeraniol and beta-citronellol were also detected. However the percent formation was very less (Fig.4).

19B.3 Accumulation of squalene in the cells grown in shake flasks

Sandalwood cell suspensions were grown in optimized medium 2 and harvestation was done after 14 days of culture. The cells were centrifuged and extracted in methanol. GC analysis of the extract showed the presence of sqaulene and farnesol. However none of the sesquiterpenes were detected in this medium (Fig. 5).

The initial leads of the work done will be continued further to improve the quantity of santalol and squalene production in the cell suspension cultures. Open reading frame (ORF) of Squalene synthase with be isolated from sandalwood and functionally characterized. The regulation of both the branch pathway genes i.e. squalene synthase and santalene synthase will be studied in response to different elicitors and best elicitor will be chosen for controlling the branch point.

17.7 Scope of the Application indicating anticipated product and processes

Santalol and squalene are versatile biomolecules with immense importance in pharma, cosmetic and fuel industry. Achieving significantly high accumulation in the cell suspension cultures of sandalwood under optimized conditions will have high potential for commercial application since the marine sources have pollution concerns, vegetal sources are limited and the compounds offers great hope in chronic diseases like cancer. Production of pharmaceutical compound from medicinally important plant looks more promising. Moreover, the system is an attractive, non-

destructive, unlimited, pollution free, safe and green alternate to the production of this important biomolecule.

18 Specific objectives:

Institute Name :Vittal Mallya Scientific Research Foundation

S.No.	Objectives
1	Optimization of medium and elicitors responsible for santalol formation in cell suspension cultures of sandalwood in shake flasks.
2	Optimization of medium and elicitors responsible for squalene formation in cell suspension cultures of sandalwood in shake flasks.
3	Isolation and functional characterization of squalene synthase gene from S. album.
4	Real time PCR expression analysis of SaSS and SaSQS in cell lines in response to different treatments and study the molecular mechanisms leading to formation of diverse compounds.

19 Workplan & Timelines for respective objectives

Institute Name :Vittal Mallya Scientific Research Foundation

Objective 1. Optimization of medium and elicitors responsible for santalol formation in cell suspension cultures of sandalwood in shake flasks.

WorkPlan 1. Culture conditions will be optimized to get the cell lines capable of santalol production without genetic manipulation. Different concentrations of elicitors will be tried and best elicitor leading to significant accumulation of santalol will be chosen for scale up studies.

Timeline

Sno.	Activity	Start Month	End Month
1	1. Optimization of medium. 2. Optimization of culture conditions. 3. Selection of best elicitor.	1	6

Objective 2. Optimization of medium and elicitors responsible for squalene formation in cell suspension cultures of sandalwood in shake flasks.

WorkPlan 1. Culture conditions will be optimized to get the cell lines capable of squalene production without genetic manipulation. Different concentrations of elicitors will be tried and best elicitor leading to significant accumulation of squalene will be chosen for scale up studies.

Timeline

Sno.	Activity	Start Month	End Month

1	4. Optimization of medium. 5. Optimization of culture conditions. 6. Selection of best elicitor.	7	12

Objective 3. Isolation and functional characterization of squalene synthase gene from S. album.

WorkPlan 1. SaSQS gene will be isolated from sandalwood and functionally characterized.

Timeline

Sno.	Activity	Start Month	End Month
1	1. Primer design. 2. Gene amplification and cloning. 3. Confirmation by sequencing.	13	18

Objective 4. Real time PCR expression analysis of SaSS and SaSQS in cell lines in response to different treatments and study the molecular mechanisms leading to formation of diverse compounds.

WorkPlan 1. Real time PCR studies will be taken up to know the molecular mechanisms involved in the formation of diverse compounds.

Timeline

Sno.	Activity	Start Month	End Month
1	1. Primer design. 2. RNA isolation. 3. cDNA synthesis. 4. Real time PCR.	19	36

20. Role and Responsibility of all Participating Investigators & Institutions

DR Arti will design the experiments, teach the SRF to perform the experiments in correct way. Dr Anil Kush will supervise the project.

21. Details of References

1. Arasada, B.L., A. Bommareddy, X.Y. Zhang, K. Bremmon and C. Dwivedi. 2008. Effects of alpha-santalol on proapoptotic caspases and p53 expression in UVB irradiated mouse skin. Anticancer Res. 28, 129-132.
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Guo Shi-Kui et al. 1983. Immediate Effect of Kuan- Xiong Aerosols in the Treament of Anginal attack. JMPR 47

22. Referances of experts in the field

SNo.	Expert Name	Designation	Address
1	Dr. Sanjay Kumar	Director, IHBT	(Director), IHBT, CSIR, Palampur (H.P.). PIN:176 061. Phone: 91-1892-233339 Ext 240. E. Mail: sanjaykumar@ihbt.res.in Fax: +91-1894-230433
2	Dr. K.K. Sharma	Director, ICRISAT	Agri Business Incubator, International Crops Research Institute for the Semi Arid Tropics, ICRISAT, 303 Bldg, Patancheru – 5023224. Phone: 040 30713071; Fax: 040 30713074. Email: k.sharma@cgiar.org
3	Dr. Manoj Prasad	Sr. Scientist	National Institute of Plant Genome Research Aruna Asaf Ali Marg, P. B. No. 10531, New Delhi 110067 (O): 91-11-26741612,14,17 Ext. 160; Direct – 011-26735160. manoj_prasad@nipgr.ac.in
4	Dr Kashmir Singh	Assistant Professor	Department of Biotechnology Panjab University, Chandigarh E. Mail- kashmirbio@pu.ac.in Mobile No- 91-9501684096
5	Dr Dharam Singh Sharma	Sr. Scientist	Plant Molecular Biology Lab CSIR, IHBT, Palampur (H.P.). PIN: 176061. Phone no.- 91-1892-233339 E. Mail- dharamsingh@ihbt.res.in

23. Uploaded figures/flowcharts/photographs referred in the project

Not uploaded

Part 4: BUDGET PARTICULARS

A. Non-Recurring (e.g. equipments, accessories, etc.)

Uploaded Quotations for Equipments

S No	Institute	Uploaded Quotation
1	Vittal Mallya Scientific Research Foundation	Not uploaded

B. Recurring

B.1 Human Resource Details

Sno.	Resource	No	Justification	Year_I	Year_II	Year_III	Total
Vittal Mallya Scientific Research Foundation							
1	Assistant Professor	1	Design, perform and execute experiments, make progress reports, presentations, manuscripts writing, UC, SOE and manpower, upload on the PFMS as well as in epromis.	936000	936000	936000	2808000
2	Project Assistant	1	Project assistant will perform experiments related to the project.	240000	240000	240000	720000
Total				1176000	1176000	1176000	3528000

B.2 Consumables

Sno.	Item	Justification	Qty	Year_I	Year_II	Year_III	Total
Vittal Mallya Scientific Research Foundation							
1	Glasswares, plasticwares, kits, chemicals	Laboratory chemicals, reagents and glasswares will be required for various experiments. Fermenter: To increase the yield of santalol and squalene fermenter runs are required time to time.	1	500000	500000	500000	1500000

Total	500000	500000	500000	1500000

B.3 Travels

Sno.	Description	Justification	Year_I	Year_II	Year_III	Total
Vittal Mallya Scientific Research Foundation						
1	Meetings will be conducted in collaborating institutions at least once in six months for project discussions and for presenting the progress of work in task force committee.	Meetings will be conducted in collaborating institutions at least once in six months for project discussions and for presenting the progress of work in task force committee.	25000	25000	25000	75000
Total			25000	25000	25000	75000

B.4 Contingency

Sno.	Description	Justification	Year_I	Year_II	Year_III	Total
Vittal Mallya Scientific Research Foundation						
1	is required to maintain instruments and plant molecular biology lab.	is required to maintain instruments and plant molecular biology lab.	50000	50000	50000	150000
Total			50000	50000	50000	150000

B.5 Overhead

Sno.	Description	Justification	Year_I	Year_II	Year_III	Total
Vittal Mallya Scientific Research Foundation						
1	To meet other expenses in the project.	To meet other expenses in the project.	100000	100000	100000	300000
Total			100000	100000	100000	300000

B.6 Recurring Others

Account Holder Details

Account Holder Name	Postal Address	Phone No	Email Id
Vittal Mallya Scientific Research Foundation			
Vittal Mallya Scientific Research	#23, 5th main, J.C. Industrial Layout, Kanakpura road,	080-268612	vmsrf@vmsrf.org

Foundation	Bangalore. PIN-560 062		

Bank Details

Account No.	Type	Bank Name	Branch Name	IFC Code	MICR Code	Phone No.
Vittal Mallya Scientific Research Foundation						
54041431074	Current	STATE BANK OF INDIA	Ifb Bangalore	SBMY0040522	560006063	8025583656

Part 5: EXISTING FACILITIES

1. Laboratory:

a. Human Resource:

VMSRF has competent staff who can guide the exclusive project staff on various techniques to be followed and in the usage of various equipments.

b. Equipments :

Centrifuge

GC MS

Gel documentation

HPLC

Incubator shaker
Laminar flow hoods

Lyophilizer

PCR Machine

Real Time PCR Machine

Spectrophotometer

c. Other resources such as clinical material, animal house facility, glass gourse, experimental:

NIL

Part 5: Biodata of Investigators

Project Investigator Details:

1) Name: Dr. Arti Rani

Designation: PI

Department: Biotechnology

Institute: Vittal Mallya Scientific Research Foundation

Date Of Birth: 26/02/1978

Sex: Female

SC/ST: No

II) Education Details:

Sno.	Institution Place	Degree Awarded	Year	Field of Study
1	H.P. University, Shimla	MSc Botany	2000	Botany
2	H.P. University, Shimla	M. Phil Botany	2002	Plant Physiology
3	IHBT, CSIR, Palampur (H.P.)	PhD Biotechnology	2008	Biotechnology specialization- Plant Molecular Biology

III) Employment Details:

Sno.	Institution Place	Position	From (Date)	To (date)
1	Vittal Mallya Scientific Research Foundation, Bangalore	Principal Investigator	19/02/2008	Till Date

IV) Honors/Awards:

Sno.	Reader	No.	Description
1.	International		
2.	National		

V) Publications:

Sno.	Reader	No.
1.	International	12
2.	National	1

Uploaded additional information **View uploaded document**

Publication Details:

Uploaded list of Publication in the peer review Journal of impact factor 1 and above

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Sno.	Title of Paper	Author	Reference of journal	Year
1	"Biofortification of Safflower - an oil seed crop engineered for ALA- targeting better sustainability and plant based omega-3 fatty acids." Accepted on April 5th, 2018 ((DOI: 10.1007/s11248-018-0070-5).	Arti rani, Asha Panwar, Manjary Sathe, Anil Kush	Transgenic research	2018

VI) Project(s) submitted/being pursued/carried out by Investigator:

Sno.	Title of Project	Funding Agency	From Date - To Date	Current Status of Project (Role)	No. of Scientists	Approved Cost
1	6. “Gene transfer and expression system studies of key Santalol pathway genes in Sandalwood cell culture system.”	DBT	12/15/2015 to 12/15/2018	Being pursued (PI)	1	3458000.00

VII) Professional Experience and Training relevant to the Project:

Part 6: DECLARATION/CERTIFICATION

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