Conservation, metabolomics, bioactivity and enhancement of secondary metabolites of Oroxylum indicum L.

File Number : SUR/2022/004759

Submitted By : Dr. Binod Kumar Mahto
Submission Date : 07-Oct-2022

PROPOSAL DETAILS

(SUR/2022/004759)

Dr. Binod Kumar Mahto

krvinod09@gmail.com

Assistant Professor (University Department Of Botany)

Ranchi University

Off kanke rd, near shaheed chowk, morabadi, ranchi, Ranchi, Jharkhand-834001

[State University (Public)]

Technical Details :

Scheme : State University Research Excellence (SERB SURE)

Research Area : Organismal and Evolutionary Biology (Plant Science) (Life Sciences)

Duration : 36 Months

Contact No : +918010140158

Date of Birth : 30-Jan-1986

Nationality : INDIAN

Total Cost (INR) : 29,55,600

Project Summary :

Oroxylum indicum L. is an important medicinal plant and enriched with secondary metabolites components like flavonoids, phenolics, alkaloids, and whole plants are widely used in Ayurveda and pharmaceutical industry to treat various disease such as asthma, dysentery, bronchitis, fever, cancer, emollient, antimicrobial, anti-inflammatory, bronchitis, leukoderma, neuroprotective and anti-eliptic. With increase the population the demand is also increases, to meet the demands, this plant is highly exploited and threatened and now came under endangered species. So, for the conservation and meet the demand several studies have been reported on O. indicum L. tissue culture technique, but till date an efficient and reproducible protocol was not developed. So, in the current proposal, we have to develop an efficient and reproducible In vitro tissue culture protocol. Respectively, to increase the commercial and pharmaceutical gain, enhance the secondary metabolites such as phenolics, flavonoids and alkaloids by using elicitor through callus or cell suspension culture. We aim to analyze the metabolomics of O. indicum L. and bioactivity studies would be developed and performed.

Objectives :

- Standardization of an in vitro multiplication of Oroxylum indicum L.
- Enhancement, Isolation and quantification of secondary metabolites
- Bioassay of the bioactive compound against bacteria and fungi
- Plantation of regenerated plants into the field/forest

Keywords :

Oroxylum indicum L., Tissue Culture, Metabolomics, Bioactivity, Conservation, Secondary Metabolites

Expected Output and Outcome of the proposal :

Following are the suggested plan of action for the utilization of the expected research outcome from the proposed study

- An efficient regeneration protocol could be utilized to mass multiplication of O. indicum L.
- Simultaneously, we will evaluate in vitro antifungal and antibacterial potential of leaf extracts against a spectrum of plant pathogens.
- Enhancement of secondary metabolites by using elicitor could be utilized the method in pharmaceutical industry.
- Germplasm conservation of O. indicum L.
- Concurrently, we will be establishing industrial collaboration to translate our product.
- Participation in world-leading conferences, publication in high-impact journals

Suitability of the proposed work in major national initiatives of the Government:

Not Applicable

Theme of Proposed Work:

Others

Collaboration Details for last 5 Years :

Planned Collaboration for the proposed work with any foreign scientist/ institution ? **No**

Other Technical Details

Conservation, metabolomics, bioactivity and enhancement of secondary metabolites of *Oroxylum indicum* L.

1. Origin of the Proposal:

Oroxylum indicum (Indian trumpet tree) is an important medicinal and small deciduous and soft wooded tree belonging to the begnoniaceae family and also known as sonapatha, soyanka or midnight horror plant. Whole plants parts possess medicinal value like root bark, seeds, leaves flower are used in asthma, dysentery, bronchitis, fever, cancer, emollient, antimicrobial, anti-inflammatory, bronchitis, leukoderma etc. and, also contains secondary metabolites such as alkaloids and flavonoids, which is used in the Ayurveda and pharmaceutical industry (Dinda et al., 2015; Samatha and Rama Swamy 2020; Nik Salleh et al., 2020). So, it's over exploitation of this valuable plants become threatened in Andhra Pradesh and Karnataka, whereas endangered in Madhya Pradesh, Kerala, Chhattisgarh and Maharashtra (Darshan and Ved 2003; Jayram and Prasad 2008; Lodh and Swamy, 2020). *O. indicum* L. has been pushed into the list of Indian red data book, due to increases its demand in pharmaceautical industry. So, for the conservation of this plant there is an urgent need for its mass multiplication via in vitro tissue culture technique such as direct or indirect organogenesis, micropropagation, embryo culture, meristem culture etc. Several investigations reported that the direct or indirect organogenesis by using leaf, somatic embryo, nodal, shoot tip culture and cotyledonary leaves explants on various combinations of phytohormones but they not achieved the shoot bud induction and regeneration efficiency was low (Dalal and Rai, 2004; Gokhle and Bansal 2009; Samatha et al. 2013; Samatha et al.2016,). So, need to develop an efficient regeneration protocol for the large sclae multiplication of *O. indicum* plant. O. indicum is an excellent source of bioactive compounds such as flavonoids (Oroxylin A, Chrysin, dihydroxy oroxylin etc.), naphthalenoids, phenolics, alkaloids, terpenoids, steroids, xanthones etc. (Sharma et al. 2022) and these compounds having antibacterial (*Staphylococcus aureus, B. subtilis, K. Pneumoniae, E. coli, S. intermedius, S. suis, C. albicans*), antifungal (dermatophore and wood rot) and antiviral (Chikungunya, Zika and Mayaro virus) properties (Ali et al. 1998; Fan et al. 2015; Yossathera et al. 2016; DeFilipps and Krupnick, 2018; Lama et al. 2022; Reis et al. 2022; Sharma et al. 2022; Sultana et al. 2022). To meet the demand of metabolites to the pharmaceutical industry, the metabolites productivity can be enhanced by

using the elicitor such as chitosan etc. through callus and cell suspension culture. Till date there is no report on callus and cell suspension culture of *O. indicum*.

So, in the current proposal, we have to develop a highly efficient *in vitro* multiplication protocol for *Oroxylum indicum L.* plants. Also aimed for enhancement of secondary metabolites like flavonoids, phenolics and steroids by using plant elicitors through callus and cell suspension culture. Quantification of enhanced metabolite by using HPLC and, in vitro bioassay bioactivity of plant extract against fungal and bacterial pathogen causing devastating disease in cultivated vegetable crops (chili and tomato). For the germplasm conservation of *O. indicum*, plantation of regenerated plants carried out to the forest and local area.

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

2.1 International Status:

Oroxylum indicum one of the important medicinal plant belongs to the family bignoneaceae and it grows in forest and distributed in Asian country such as India, China, Thialand, Nepal, Indonesia, Malaysia, Philippines, Sri Lanka and Cambodia. The whole plants parts like leaves, stem bark, flowers, seeds, fruits and roots are used as traditional medicine to treat various diseases (respiratory tract, asthma, dysentery, bronchitis, fever, cancer, emollient, antimicrobial, anti-inflammatory, bronchitis, leukoderma etc.). It contains high value of secondary metabolites as well as having antioxidant activity. Chi et al. (2012), reported that plant stem bark used as a traditional medicine to treat hepatitis, cough, bronchitis, sore throat and psoriasis. Anja and Markus (2012), analysed flavonoids by using HPLC-MS from seeds extract of *O. indicum* and these seeds were therapeutic used against infectious disease of the respiratory tract. Whereas, Nguyen et al. (2012), investigated inhibitory activity of glucosidase by using extracts of the heartwood of *O. indicum*. *In vitro* antioxidant potency of three flavones such as chrysin, oroxylin A and baicalein from stem bark extract by using DPPH assay (Trang et al. 2018). Later on, Le et al. (2020), isolated and reported two novel metabolites oroxindols A and B from stem bark of *O. indicum*. Recently, examine the effect of *O. indicum* on cognitive function in older adults and observed that greater improvement in episodic memory, and also analysed the changes in concentration of brain derived neurotrophic factors (Lopresti et al. 2021).

2.2 National Status:

In India, Ayurveda Chyawanprasha and Dashamoolarishta containing O. *indicum* component and its compound (Dev et al. 2010) and these compounds such as flavonoids, alkaloids, tannins, glycosides, saponins, phenols, and quinones were extracted from different parts of the plants (Dinda et al., 2015; Nik Salleh et al., 2020). Among these flavonoids one of the major storage component that includes baicalein, baicalein-7-O-diglucoside, chrysin, and oroxylin-A (Chen et al., 2003; Yadav et al., 2013). These compounds having several bioactivity such as anticancer, antimicrobial, antioxidants, anti-inflammatory, analgesic, antihyperglycemic, neuroprotective and anti-eliptic (Dinda et al., 2015; Rathod 2016; Nik Salleh et al., 2020). *O. indicum* plants are highly exploited and threatened medicinal plants due to its high medicinal value. So, plant tissue culture technique was used to increase the raw material as well as increase the production of bioactive components of the plant. Few reports found that the highest

amounts of total phenolics and flavonoids content in callus culture as compared to the root growing stage, cotyledon stage and leaf growing stages. Several investigations reported that the direct or indirect organogenesis by using leaf, somatic embryo, nodal, shoot tip culture and cotyledonary leaves explants on various combinations of phytohormones but they not achieved the shoot bud induction and regeneration efficiency was low (Dalal and Rai, 2004; Tiwari et al. 2007; Gokhle and Bansal 2009; Samatha et al. 2013; Rami and Patel, 2014; Samatha et al.2016).

2.3 Importance of the proposed project in the context of current status

Oroxylum indicum L. is an important medicinal and endangered plant. So, in the current study, we have to establishment of an efficient regeneration protocol for Oroxylum indicum L. plants to conserve the germplasm. To meet the demand of pharmaceautical industry, we also aimed to enhance the secondary metabolites by using elicitors through callus and cell suspension culture. In the agricultural context, antifungal and antibacterial activity of *O. indicum* L. will be analysed by using plant extract against these pathogens to protect the vegetable crops from various disease.

2.4 If the project is location specific, basis for selection of location be highlighted:

The project is not location specific. However, all the research activities will be conducted and carried out in Ranchi and at University Department of Botany, Ranchi University, Ranchi.

3. Work Plan:

3.1 Methodology:

***In Vitro* Multiplication and Enhancement of Secondary Metabolites**: Plant Material were collected from Birsa Agriculture University, Ranchi and maintained in the Botanical garden of University Department of Botany, Ranchi University, Ranchi (Fig.1).

For the *in vitro* multiplication, leaves, internode and axillary bud will be used as an explant to develop a highly efficient regeneration protocol for *Oroxylum indicum L*. For the regeneration, we have to optimize with the various type of phytohormones combinations and concentration particularly of cytokinins and auxins in plant growth medium (MS Medium), and the culture conditions light and temperature.

In vitro enhancement and large scale production of secondary metabolites by using various elicitator such as chitosan, polyamines, Salicylic acid etc. to compare the effect of elicitor in the production of secondary metabolites through callus and cell suspension culture.

Fig. 1. *Oroxylum indicum* L. plant planted and maintained in the Botanical Garden of University Department of Botany, Ranchi University, Ranchi

Isolation and Quantification of Secondary Metabolites: To determination of contents, sample will be prepared from callus tissue, leaves of regenerated and wild-type plants of *O. indicum* using methanol extract methods, and analyze by using high-performance liquid

chromatographic technique (Tao et al. 2006). The validated HPLC analytical method was applied to undertake a quantitative analysis of metabolites in the extracts from the *O. indicum* samples. System suitability of the analytical method, including the tailing factor, theoretical plate, %RSD of the peak area, and the SD of the retention time, will be investigated.

Bioassay of bioactive compouds against bacterial and fungi: Extraction of essential oils from these plants and *in vitro* antifungal and antimicrobial activity will be conducted against fungal and bacterial pathogen, which causing devastating disease in many cultivated vegetable crops.

3.2 Time Schedule of activities giving milestones through BAR diagram.

The deliveries and milestones of this 3-years project, as summarized in the BAR chart below, alongside the objectives/activities and task breakdown. Simultaneously, we will be establishment of industrial collaboration to translate our product. Also, participation in world-leading conferences, publication in high impact journals and application for grants to expand my group activities.

Objectives/ activities	**Year 1**				**Year 2**				**Year 3**			
	Q1	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Objective 1: Standardization of an *in vitro* multiplication of *Oroxylum indicum*												
Objective 2: Enhancement, Isolation and quantification of secondary metabolites												
Objective 3: Bioassay of bioactive compound against bacteria and fungi												
Objective 4: Plantation of regenerated plants into the field/forest												

3.3 Suggested Plan of action for utilization of research outcome expected from the project.

Following are the suggested plan of action for utilization of expected research outcome from the proposed study

- An efficient regeneration protocol could be utilized to mass multiplication of O. indicum L.
- Simultaneously, we will evaluate *in vitro* antifungal and antibacterial potential of leaf extracts against a spectrum of plant pathogens.
- Enhancement of secondary metabolites by using elicitor could be utilized the method in pharmaceutical industry.
- Germplasm conservation of *O. indicum* L.
- Concurrently, we will be establishment of industrial collaboration to translate our product.
- Participation in world-leading conferences, publication in high impact journals

3.4 Environmental impact assessment and risk analysis.

There are no environmental risks expected in conducting the proposed project activities.

4. Expertise:

4.1 Expertise available with the investigators in executing the project:

The PI has been trained in major tools of plant tissue culture and plant molecular biology and has over 10 years of research experience after post-graduation in this area of research including PhD thesis work.

4.2 Summary of roles/responsibilities for all Investigators:

Proposal is single institutional and involved one PI. The PI will be responsible for overall research planning and coordination, analysis of results and communications with SERB and publications.

4.3 Key publications published by the Investigators pertaining to the theme of the proposal during the last 5 years

- Sagar S Arya, **Binod Kumar Mahto**, Meenu Senger, Jim E Rookes, David M Cahill, Sangram K Lenka. **Metabolic engineering of rice cells with vanillin synthase gene (*VpVAN*) to produce vanillin (2022)**. *Molecular Biotechnology*, Springer Nature. (https://doi.org/10.1007/s12033-022-00470-8).

- Nidhi Dongre, Divyani Kumari, **Binod Kumar Mahto**, Sagar Sanjay Arya, Ashraf Saifullah, Sangram Keshari Lenka. **Mutagenomics for functional analysis of *plant genome using CRISPR* library screen (2021)**. *RNA-Based Technologies for Functional Genomics in Plants*, Springer Nature, pp 339-367. (DOI: 10.1007/978-3-030-64994-4).
- **Binod kumar Mahto**, Anjulata Singh, Manish Pareek, M V Rajam, Swatismita Dhar-Ray, P M Reddy. **Host-induced gene silencing of *conidial morphology 1* gene (*CgCOM1*) to confers resistance against anthracnose in chilli and tomato (2020)**. *Plant Molecular Biology*, Springer Nature; 104:381-395. (PMID: 32803478, DOI: 10.1007/s11103-020-01046-3).
- Sagar S Arya, **Binod Kumar Mahto**, Thakku R Ramkumar, Sangram K Lenka. **Sharpening gene editing toolbox in Arabidopsis for plants (2020)**. *Journal of Plant Biochemistry and Biotechnology*, Springer nature, 29: 769-784. (DOI: 10.1007/s13562-020-00606-4).
- **Binod kumar Mahto**, Amit Katiyar, Sangram K Lenka, Kailash C Bansal. **Small RNA technology for plants abiotic stress tolerance (2020)**. *Plant small RNA: Biogenesis, Regulation and application, 1st edition, Acedamic press*: Elsevier, Pages 521-541. (doi.org/10.1016/B978-0-12-817112-7.00023-7).
- **Binod kumar Mahto**, Poonam Sharma, M V Rajam, P M Reddy, Swatismita Dhar-Ray. **An efficient method for *Agrobacterium*-mediated genetic transformation of chilli pepper (*Capsicum annuum* L) (2018)**. *Plant Physiology Repots* (Formerly *Indian Journal of Plant Physiology)*, Springer Nature, 23:573-581. (https://doi.org/10.1007/s40502-018-0389-1.

4.4 Bibliography

Ali RM, Houghton PJ, Hoo TS (1998). Antifungal activity of some Bignoniaceae found in Malaysia, *Phytotherapy Research*, **12**(5): 331–4.

Anja K, Markus G (2012). Oroxylum indicum seeds – Analysis of flavonoids by HPLC–MS, Journal of Pharmaceutical and Biomedical Analysis, 70 : 553-556

Chen LJ, Games DE, Jones J (2003). Isolation and identification of four flavonoid constituents from the seeds of *Oroxylum indicum* by high-speed counter-current chromatography. *J. Chromatogr. A.* 988, 95–105. doi: 10.1016/s0021-9673(02)01954-4

Dalal NV, Rai VR (2004). In vitro propagation of Oroxylum indicum Vent. a medicinally important forest tree. *Journal of Forest Research* 9.1: 61-65.

DeFilipps RA, Krupnick GA (2018). The medicinal plants of Myanmar. PhytoKeys 102: 1-341. https://doi.org/10.3897/phytokeys.102.24380

Dinda B, Silsarma I, Dinda M, Rudrapaul P (2015). *Oroxylum indicum* (L.) Kurz, an important asian traditional medicine: from traditional uses to scientific data for its commercial exploitation. *J. Ethnopharmacol.* 161, 255–278. doi: 10.1016/j.jep.2014.12.027

Gokhale M, Bansal YK (2009). Direct in vitro regeneration of a medicinal tree *Oroxylum indicum* (L.) Vent. through tissue culture. Afr J Biotechnol 8:3777–3781

Gokhale M, Bansal YK (2009). Direct in vitro regeneration of a medicinal tree Oroxylum indicum (L.) Vent. through tissue culture. *African Journal of Biotechnology*, *8*(16).

Jasrai YT, Kalpana NT, Parmar VR (2013). "Propagation of Oroxylum indicum (L.) Vent, a vulnerable medicinal tree through organogenesis." *Plant Tissue Culture and Biotechnology* 23.1: 127-132.

Le TV, Tran THH, Tran HQ, Nguyen TC, Nguyen XC, Hyuncheol O, Nguyen VS, Nguyen HN, Chau VM (2020). Oroxindols A and B, two novel secoabietane diterpenoids from Oroxylum indicum, Phytochemistry Letters; 40: 101-104.

Lodh, S, Swamy, MK (2020). *Oroxylum indicum* Vent. and Its Bioactive Compound, Baicalein Against Cancer Cells: Mechanisms of Action. In: Swamy, M. (eds) Plant-derived Bioactives. Springer, Singapore. https://doi.org/10.1007/978-981-15-2361-8_20

Lopresti AL, Smith SJ, Majeed M and Drummond PD (2021). Effects of an Oroxylum indicum Extract (Sabroxy®) on Cognitive Function in Adults With Self-reported Mild Cognitive Impairment: A Randomized, Double-Blind, Placebo-Controlled Study. Front. Aging Neurosci. 13:728360. doi: 10.3389/fnagi.2021.728360

Mabhavi R, Kamdi SR, Wadhai VS (2011). "In vitro shoot induction and callus induction of a medicinal tree Oroxylum indicum (Tattu) through tissue culture." *International Journal of Plant Sciences (Muzaffarnagar)* 6.1: 45-48.

Nguyen MTT, Nguyen NT, Nguyen HX, Huynh TNN, Min BS (2012). Screening of α-Glucosidase Inhibitory Activity of Vietnamese Medicinal Plants : Isolation of Active Principles from Oroxylum indicum, Natural Product Sciences; 18 (1): 47-5.

Nik Salleh NNH, Othman FA, Kamarudin NA, Tan SC (2020). The biological activities and therapeutic potentials of baicalein extracted from *Oroxylum indicum*: a systematic review. *Molecules* 25:5677. doi: 10.3390/molecules25235677

Qing-Fei Fan, Zu-Yan Hu, Zhi Na, Hua-Shu Tang, Guo-Ying Zuo & Qi-Shi Song (2015). One new flavonoid from *Oroxylum indicum*, Natural Product Research, 29:19, 1828-1832, DOI: 10.1080/14786419.2015.1007976

Rami E, Patel I (2014). Effect of growth regulators and explant types on callus induction from Oroxylum indicum (L.) Vent. *Life Science* 1.12: 278-280.

Rathod KR, Anandaapara RC, Karla MJ, Ganatra TH (2016). Evaluation of effect of *Oroxylum indicum* leaves on central nervous system with special emphasis on epilepsy. *J. Chem. Pharm. Res.* 8, 680–685.

Samatha T, Rama Swamy, N (2020). Conservation of an Endangered Medicinal Forest Tree Species, *Oroxylum indicum* L. Kurz, Through In Vitro Culture: A Review. In: Khasim, S.M., Long, C., Thammasiri, K., Lutken, H. (eds) Medicinal Plants: Biodiversity, Sustainable Utilization and Conservation. Springer, Singapore. https://doi.org/10.1007/978-981-15-1636-8_47

Sithisarn P, Rojsanga P, Sithisarn P (2019). Inhibitory effects on clinical isolated bacteria and simultaneous HPLC quantitative analysis of flavone contents in extracts from Oroxylum indicum. Molecules, 24, 1937

Tiwari S, Singh K, Shah P (2007). In vitro propagation of Oroxylum indicum-An endangered medicinal tree. *Biotechnology* 6.2 : 299-301.

Trang DHT, Son, Le H, Trung P V (2018). Investigation on the in vitro antioxidant capacity of methanol extract, fractions and flavones from Oroxylum indicum Linn bark. Brazilian Journal of Pharmaceutical Sciences [online], 54 : 01

Yadav AK, Manika N, Bagchi GD, Gupta MM (2013). Simultaneous determination of flavonoids in *Oroxylum indicum* by RP-HPLC. *Med. Chem. Res.* 22, 2222–2227. doi: 10.1007/s00044-012-0214-8

5. List of Projects submitted/implemented by the Investigators: NILL

5.1 Details of Projects submitted to various funding agencies:

S. No	Title	Cost in Lakh	Month of submission	Role as PI/Co-PI	Agency	Status

5.2 Details of Projects under implementation

S. No	Title	Cost in Lakh	Duration	Role as PI/Co-PI	Agency

5.3 Details of Projects completed during the last 5 years

S. No	Title	Cost in Lakh	Duration	Role as PI/Co-PI	Agency

6. List of facilities being extended by parent institution(s) for the project implementation.

6.1 Infrastructural Facilities

Sr. No.	Infrastructural Facility	Yes/No/ Not required Full or sharing basis
1.	Workshop Facility	Not Required
2.	Water & Electricity	YES
3.	Laboratory Space/ Furniture	YES
4.	Power Generator	YES
5.	AC Room or AC	YES
6.	Telecommunication including e-mail & fax	YES
7.	Transportation	Not Required
8.	Administrative/ Secretarial support	YES
9.	Information facilities like Internet/Library	YES
10.	Computational facilities	YES
11.	Animal/Glass House	NO
12.	Any other special facility being provided	NO

6.2 Equipment available with the Institute/ Group/ Department/Other Institutes for the project:

Equipment available	**Generic Name of Equipment**	**Model, Make & year of purchase**	**Remarks including accessories available and current usage of equipment**
PI & his group	Nil	-	-
PI's department (Central Instrumentation Lab)	Thermal Cycler	Eppendorf, 2019	100%
	Microcentrifuge	Eppendorf, Model No. 5418R, 2019	100%
	-80° deep freezer	Eppendorf, U410862019	100%
	Orbital shaker/incubator	2019	100%
	Electronic balances	2019	
	Submarine gel electrophoresis system	Eppendorf, 2019	100%
	Spectrophotometer	Eppendorf,2019	100%
	Chemiluminescence imaging system	Eppendorf,2019	100%
	Vortex	Eppendorf, 2019	100%
	Fermentor	Eppendorf BIOFLO-120,205LPM, 2019	100%
	pH Meter		100%
	Image Analyser	Thermo Scientific	100%
Other Institute(s) in the region			

7. Name and address of experts/ institution interested in the subject / outcome of the project.

8. Previous Projects Details (If Any): NILL

S. No	Project Title	PI Name	CO-PI Name	Amount	Status	Date Of Start	Date Of Completion	Funding Agency

Budget Details

Institution wise Budget Breakup :

Budget Head	Ranchi University	Total
Research Personnel	8,49,600	**8,49,600**
Consumables	9,00,000	**9,00,000**
Travel	1,50,000	**1,50,000**
Equipment	5,56,000	**5,56,000**
Contingencies	2,00,000	**2,00,000**
Other cost	1,00,000	**1,00,000**
Overhead	2,00,000	**2,00,000**
Total	**29,55,600**	**29,55,600**

Institute Name : *Ranchi University*

Year Wise Budget Summary (Amount in INR) :

Budget Head	Year-1	Year-2	Year-3	Total
Research Personnel	2,83,200	2,83,200	2,83,200	**8,49,600**
Consumables	4,00,000	2,50,000	2,50,000	**9,00,000**
Travel	50,000	50,000	50,000	**1,50,000**
Equipments	5,56,000	0	0	**5,56,000**
Contingencies	1,00,000	50,000	50,000	**2,00,000**
Other cost	50,000	50,000	0	**1,00,000**
Overhead	80,000	60,000	60,000	**2,00,000**
Grand Total	**15,19,200**	**7,43,200**	**6,93,200**	**29,55,600**

Research Personnel Budget Detail (Amount in INR) :

Designation	Year-1	Year-2	Year-3	Total
Project Assistant *To complete research wet lab work such as in vitro propagation, bioassay, and metabolomics analysis. We required one Project Assistant.*	2,83,200	2,83,200	2,83,200	**8,49,600**

Consumable Budget Detail (Amount in INR) :

Justification	Year-1	Year-2	Year-3	Total
To achieve the proposed objectives we need various reagents, chemicals, plasticware, and glassware.	4,00,000	2,50,000	2,50,000	**9,00,000**

Travel Budget Detail (Amount in INR) :

Justification (Inland Travel)	Year-1	Year-2	Year-3	Total
Local travel is needed to collect plant material, and attend conferences, local meetings, project activities, workshops, and training events and plantations in the forest or local areas.	50,000	50,000	50,000	**1,50,000**

Equipment Budget Detail (Amount in INR) :

Generic Name ,Model No. , (Make)/ Justification	Quantity	Spare time	Estimated Cost
Minus 20 Deep Freezer Bluestar (Bluestar, India) *To maintain the various fluids, lower chemicals, and biological activity in a controlled environment (refrigerated space), so that they are kept in good condition the lower the temperature.*	1	50 %	**60,000**
Laminar Air Flow Laminar Air flow Horizontal ÁCCO brand (ACCO, USA) *For the In Vitro multiplication of the plants.*	1	50 %	**1,50,000**
WEIGHING BALANCE BRAS 224 (BR Biochem, India) *For the weighing of chemical powders.*	1	50 %	**60,000**
Refrigrator 4 degree Blue Star (Blue Star) *To maintain the various fluids, lower chemicals, and biological activity in a controlled environment (refrigerated space), so that they are kept in good condition the lower the temperature.*	1	0 %	**25,000**
Orbital Shaker Incubator REMI RIS 24 Plus Orbital Shaking Incubator (REMI, India) *Extraction of secondary metabolites and suspension culture.*	1	50 %	**2,00,000**
Pipettes Rainin Mettler Toledo Pipettes (Mettler Toledo) *A tool used to dispense measured volumes of liquids.*	1	0 %	**61,000**

Contingency Budget Detail (Amount in INR) :

Justification	Year-1	Year-2	Year-3	Total
As per the government rule, contingency grants will be used for the research lab like stationery, accessories, and registration fees for conferences.	1,00,000	50,000	50,000	**2,00,000**

Overhead Budget Detail (Amount in INR) :

Justification	Year-1	Year-2	Year-3	Total
Overhead expenses for the institute including infrastructural facilities and laboratory.	80,000	60,000	60,000	**2,00,000**

Other Budget Detail (Amount in INR) :

Description/Justification	Year-1	Year-2	Year-3	Total
Outsoursing For the quantification of secondary metabolites	50,000	50,000	0	**1,00,000**

BIO-DATA

1. Name and full correspondence address: **Dr Binod Kumar Mahto**
Assistant Professor
University Department of Botany
Basic and Applied Science Building
Ranchi University Ranchi
Morabadi, Ranchi
Jharkhand- 834008, India

2. Email(s) and contact number(s): **krvinod09@gmail.com; +91-8010140158**

3. Institution: **Ranchi University, Ranchi, Jharkhand, India**

4. Date of Birth: **30 January 1986**

5. Gender (M/F/T): **Male**

6. Category Gen/SC/ST/OBC: **OBC**

7. Whether differently abled (Yes/No): **No**

8. Academic Qualification (Undergraduate Onwards)

	Degree	Year	Subject	University/Institution	% of marks
1.	**B.Sc.**	**2009**	**Botany**	**Ranchi University, Ranchi, India**	**65.13**
2.	**M.Sc.**	**2011**	**Botany**	**Ranchi University, Ranchi, India**	**74.25 (Gold Medalist)**
3.	**P.G.D.M.P.**	**2012**	**Ethnobotany**	**Ranchi University, Ranchi, India**	**78.80 (First Rank)**
4.	**Ph.D.**	**2019**	**Plant Biotechnology**	**TERI School of Advanced Studies, New Delhi, India**	**Awarded**

9. Ph.D thesis title: **Development of transgenic lines of tomato and chilli plants against anthracnose disease**

Guide's Name: **Dr. Pallavolu Maheswara Reddy**

Institute/Organization/University: **TERI School of Advanced Studies, New Delhi, India**

Year of Award: **2019**

10. Work experience (in chronological order).

S.No.	Positions held	Name of the Institute	From	To	Pay Scale
1.	Assistant Professor	Ranchi University, Ranchi	Nov-2020	Till Date	57700/- To 182400/-

11. Professional Recognition/ Award/ Prize/ Certificate, Fellowship received by the applicant.

S.No	Name of Award	Awarding Agency	Year
1.	**Gold Medal**	**Ranchi University, Ranchi, India**	**2011**
2.	**Teaching Assistantship Award**	**Ranchi University, Ranchi, India**	**2012**
2.	**DST-INSPIRE Junior Research Fellowship**	**Department of Science and Technology, Government of India, New Delhi, India**	**2013**
3.	**DST-INSPIRE Senior Research Fellowship**	**Department of Science and Technology, Government of India, New Delhi, India**	**2015**
4.	**Best Poster Award**	**Indian Phytopathological Society, New Delhi, India**	**2016**
5.	**Best Speaker Award**	**Nineteenth International Conference on Molecular Plant-Microbe Interactions, Osaka, Japan**	**2017**
6.	**Travel Grant**	**Department of Biotechnology, Govt. of India**	**2017**
7.	**Young Researcher Award**	**Institute of Scholars**	**2021**

12. Publications *(List of papers published in SCI Journals, in year wise descending order).*

S.No.	Author(s)	Title	Name of Journal	Volume	Page	Year
1.	Sagar S Arya, **Binod Kumar Mahto**, Meenu Sengar, Jim E Rookes, David M Cahill, Sangram Lenka	Metabolic engineering of rice cells with vanillin synthase gene (*VpVAN*) to produce vanillin	*Molecular Biotechnology, Springer Nature*	64	861-872	2022
2.	**Binod kumar Mahto**, Anjulata Singh, Manish Pareek, M V Rajam, Swatismita Dhar-Ray, P M Reddy	Host-induced gene silencing of *conidial morphology 1* gene (*CgCOM1*) to confers resistance against anthracnose in chilli and tomato	*Plant Molecular Biology, Springer Nature*	104	381–395	2020
3.	Sagar S Arya, **Binod Kumar Mahto**, Thakku R Ramkumar, Sangram K Lenka	Sharpening gene editing toolbox in Arabidopsis for plants	*Journal of Plant Biochemistry and Biotechnology, Springer Nature*	29	769–784	2020
4.	**Binod kumar Mahto**, Poonam Sharma, M V Rajam, P M Reddy, Swatismita Dhar-Ray	An efficient method for *Agrobacterium*-mediated genetic transformation of chilli pepper (*Capsicum annuum* L)	*Plant Physiology Reports (Previously Indian Journal of Plant Physiology), Springer Nature*	23	573-581	2018

13. Detail of patents.

S.No	Patent Title	Name of Applicant(s)	Patent No.	Award Date	Agency/Country	Status

14. Books/Reports/Chapters/General articles etc.

S.No	Title	Author's Name	Publisher	Year of Publication
1.	Small RNA technology for plants abiotic stress tolerance	**Binod kumar Mahto**, Amit Katiyar, Sangram K Lenka, Kailash C Bansal	*Elsevier*	2020
2.	Mutagenomics for Functional Analysis of Plant Genome Using CRISPR Library Screen	Nidhi Dongre, Divyani Kumari, **Binod Kumar Mahto**, Sagar Sanjay Arya, Sangram Keshari Lenka	*Springer Nature*	2021

15. Any other Information (maximum 500 words):

Certificate from the Investigator

Project Title: Conservation, metabolomics, bioactivity and enhancement of secondary metabolites of *Oroxylum indicum* L.

It is certified that

1. The same project proposal has not been submitted elsewhere for financial support.

2. We/I undertake that spare time on equipment procured in the project will be made available to other users.

3. We/I agree to submit a certificate from Institutional Biosafety Committee, if the project involves the utilization of genetically engineered organisms. We/I also declare that while conducting experiments, the Biosafety Guidelines of Department of Biotechnology, Department of Health Research, GOI would be followed in toto.

4. We/I agree to submit ethical clearance certificate from the concerned ethical committee, if the project involves field trails/experiments/exchange of specimens, human & animal materials etc.

5. The research work proposed in the scheme/project does not in any way duplicate the work already done or being carried out elsewhere on the subject.

6. We/I agree to abide by the terms and conditions of SERB grant.

15/9/2022

Name and signature of Principal Investigator: **Dr. Binod Kumar Mahto**

Date:

Place:

Dr. Binod Kumar Mahto
Assistant Professor
University Deptt. of Botany
Ranchi University, Ranchi

Name and signature of Co-PI (s) (if any):

Date:

Place:

Undertaking by the Principal Investigator

To

The Secretary
SERB, New Delhi

Sir

I DR. BINOD KUMAR MAHTO herby certify that the research proposal titled Conservation, Metabolomics, bioactivity and enhancement of secondary Metabolites of Oroxylum indicum L. submitted for possible funding by SERB, New Delhi is my original idea and has not been copied/taken verbatim from anyone or from any other sources. I further certify that this proposal has been checked for plagiarism through a plagiarism detection tool i.e. Turnitin approved by the Institute and the contents are original and not copied/taken from any one or many other sources. I am aware of the UGCs Regulations on prevention of Plagiarism i.e. University Grant Commission (Promotion of Academic Integrity and Prevention of Plagiarism in Higher Educational Institutions) Regulation, 2018. I also declare that there are no plagiarism charges established or pending against me in the last five years. If the funding agency notices any plagiarism or any other discrepancies in the above proposal of mine, I would abide by whatsoever action taken against me by SERB, as deemed necessary.

Signature of PI with date

Name / designation

Dr. Binod Kumar Mahto
Assistant Professor
University Deptt. of Botany
Ranchi University, Ranchi

Certificate from the Investigator

Project Title: **Conservation, metabolomics, bioactivity and enhancement of secondary metabolites of *Oroxylum indicum* L.**

It is certified that

1. The same project proposal has not been submitted elsewhere for financial support.

2. We/I undertake that spare time on equipment procured in the project will be made available to other users.

3. We/I agree to submit a certificate from Institutional Biosafety Committee, if the project involves the utilization of genetically engineered organisms. We/I also declare that while conducting experiments, the Biosafety Guidelines of Department of Biotechnology, Department of Health Research, GOI would be followed in toto.

4. We/I agree to submit ethical clearance certificate from the concerned ethical committee, if the project involves field trails/experiments/exchange of specimens, human & animal materials etc.

5. The research work proposed in the scheme/project does not in any way duplicate the work already done or being carried out elsewhere on the subject.

6. We/I agree to abide by the terms and conditions of SERB grant.

15/9/2022

Name and signature of Principal Investigator: **Dr. Binod Kumar Mahto**

Date:

Place:

Dr. Binod Kumar Mahto
Assistant Professor
University Deptt. of Botany
Ranchi University, Ranchi

Name and signature of Co-PI (s) (if any):

Date:

Place:

Ranchi University
Ranchi

Endorsement from the Head of the Institution of PI

(To be given on University/ Institute/Organization/College Letter head)

This is to certify that: Institute welcomes participation of Name : **Dr. Binod Kumar Mahto** Designation : **Assistant Professor, University Department of Botany, Ranchi University, Ranchi** as the Principal Investigator and .....as the Co- Investigator/s for the project titled **"Conservation, metabolomics, bioactivity and enhancement of secondary metabolites of *Oroxylum indicum* L."** and that in the unforeseen event of discontinuance by the Principal Investigator, the Co-Investigator will assume the responsibility of the fruitful completion of the project with the approval of SERB.

1. The PI, **Dr. Binod Kumar Mahto** is a permanent or regular employee of this Institute/University/Organization and has **29** years of regular service left before superannuation

2. The project starts from the date on which the University/Institute/ Organization/College receives the grant from SCIENCE & ENGINEERING RESEARCH BOARD (SERB), New Delhi.

3. The investigator will be governed by the rules and regulations of University/ Institute/Organization/College and will be under administrative control of the University/ Institute/Organization/College for the duration of the project.

4. The grant-in-aid by the SCIENCE & ENGINEERING RESEARCH BOARD (SERB), New Delhi will be used to meet the expenditure on the project and for the period for which the project has been sanctioned as mentioned in the sanction order.

5. No administrative or other liability will be attached to SCIENCE & ENGINEERING RESEARCH BOARD (SERB), New Delhi at the end of the project.

6. The University/Institute/Organization/College will provide basic infrastructure and other required facilities to the investigator for undertaking the research project.

7. The University/ Institute/Organization/College will take into its books all assets created in the above project and its disposal would be at the discretion of SCIENCE & ENGINEERING RESEARCH BOARD (SERB), New Delhi.

8. The University/ Institute/Organization/College assumes to undertake the financial and other management responsibilities of the project.

29.09.22

Seal of

University/ Institute/Organization/College

Date:

Signature

Registrar of University/Head of the Institute/
Head of organization / Principal of College

REGISTRAR
RANCHI UNIVERSITY, RANCHI