Progress Report for R&D Projects [Year 04]* FINAL

Section-A: Project Details

A1. Project Title: Enhanced production of anticancer flavones- Baicalein, Chrysin and Oroxylin A, from *Oroxylum indicum* tissue culture and studies on its chemopreventive activities.

A2. DBT Sanction Order No. & Date: BT/PR25000/NER/95/950/2017 Dated 02/09/2019

A3. Name of Principal Investigator: Dr. J. P. Saikia

Name of Co-PI/Co-Investigator: Prof. A. Ramteke

A4. Institute: Tezpur University

A5. Address with Contact Nos. (Landline & Mobile) & Email: Department of Molecular Biology and Biotechnology, Napaam, Tezpur-784028, Assam. Email: jyotizone@gmail.com, Mobile: 9957571281.

A6. Total Cost: Rs. 26,07,192/- (Rupees Twenty Six Lakhs Seven Thousand One Hundred and Ninety Two Only), Tezpur University total.

A7. Duration: 3 years

Approved Objectives of the Project:

1. Collection of plant specimens using GPS and submission of passports to NBPGR-Shillong.
2. Extraction, bioassay guided fractionation and purification of anticancer compounds from in vivo plant specimens.
3. Studies of in vitro culture and in vivo isolated anticancer compounds on standard cancer cell lines.

A9. Specific Recommendations made by the Task Force (if any):

Not any

Section-B: Scientific and Technical Progress

B1. Progress made against the Approved Objectives, Targets & Timelines during the Reporting Period:

Objectives	**Work done 2020-21**	**Work done 2021-22**	**Work done 2022-23**
1.Collection of plant specimens using GPS and submission of passports to NBPGR-Shillong.	Completed and received 10 IC no. from National Bureau of Plant Genetic Resources (ARIS cell) Pusa Campus, New Delhi(NBPGR) were received as 1. IC-0639395 SEEDS 2. IC-0639396 SEEDS 3. IC-0639397 SEEDS 4. IC-0639398 SEEDS 5. IC-0639399 SEEDS 6. IC-0639400 SEEDS 7. IC-0639401 SEEDS 8. IC-0639402 SEEDS 9. IC-0639403 SEEDS 10. IC-0639404 SEEDS A simple seed germination technique is also established following water imbibition and then transfer to compost inside the growth chamber.	**(proposal tenure 0-6 month).**	
2.Extraction, bioassay	Compound purification method has been	The compound purification from	**(proposed tenure 0-24 months)**

guided fractionation and purification of anticancer compounds from in vivo plant specimens.	standardized and all three compounds (baicalin, chrysin and oroxylin A) are purified from the root bark of *O. indicum* using extraction, fractionation, column chromatography and HPLC. Characterization by comparing with standard compounds in TLC (Rf and color under UV) and HPLC (retention time). Approximate yield of 0.34 µg, 1.94 µg, 21.55 µg of baicalein, chrysin and oroxylin A respectively in per g dry weight of root bark.	samples was repeated again. Characterization with respect to FTIR and quantification using UV-Vis spectrophotometer is carried out. Antioxidant activity and antibacterial activity of the purified compound are tested. As there is a difficulty in obtaining hairy roots by *O. indicum* transformation a new approach is carried out to create hairy roots in hydroponic condition with IAA and IBA treatment.	
3.Studies of in vitro culture (plant tissue cultured) and in vivo isolated anticancer compounds on standard cancer cell lines.	Commercially purchased pure compounds are tested for anticancer activity with existing standard methods and concentration (10, 25, 50 and 100 mM). The compound concentration may have to be increased to obtain a significant anticancer activity against MDA-MB-231 breast cancer cell line..	The three pure compounds with anticancer activity have been tested at higher concentration (100, 200, 300, 400 and 500 mM) with significant anticancer activity against MDA-MB-231 breast cancer cell lines. The purified compounds were also tested for anticancer activity **(proposed tenure 18-36 months)**.	Germination of the *O. indicum* seed in the laboratory and hairy root induction by hormone (IAA and IBA) in hydroponic and harvesting the 60 days old plant for compound purification (baicalin, chrysin and oroxylin A). Isolation, identification and quantification of compounds (baicalin, chrysin and oroxylin A) HPLC, FTIR, LC-MS and UV-Vis methods from germinated samples mentioned above. Compound isolation, identification and quantification was also carried out from hairy root and leaf samples of laboratory grown plants of *O. indicum* as received from ADP

			college (collaborator), infected root with *Agrobacterium rhizogene* (MTCC-532). **(proposed tenure 18-36 months)**.

Objective1: Plant Sample Collection

Sample for compound purification

Different plant parts were collected from Tezpur, Lakhimpur and Arunachal Pradesh. *O. indicum* seeds pods were collected from 10 sites of Tezpur and the morphological details of the plants, seeds pods and seeds were collected. The seeds were collected from the seed pods and we have obtained IC number from the National Bureau of Plant Genetic Resources (ARIS cell) Pusa Campus, New Delhi(NBPGR) for the seeds. Plant roots and stem barks were collected from Tezpur, Arunachal Pradesh and Nagaon. The roots and barks were dried and grinded to powder and were used for the preparation of methanolic extract. The extract was then further fractionated for the isolation of baicalein, chrysin and oroxylin A. Morphological records suggest the plant can reach 35 feet in height (Figure1, C), 2-4 feet in width at base, 50-100 pods (flat dagger like approximately 71x7 cm in dimension) per tree, 500-600 seeds per pod.

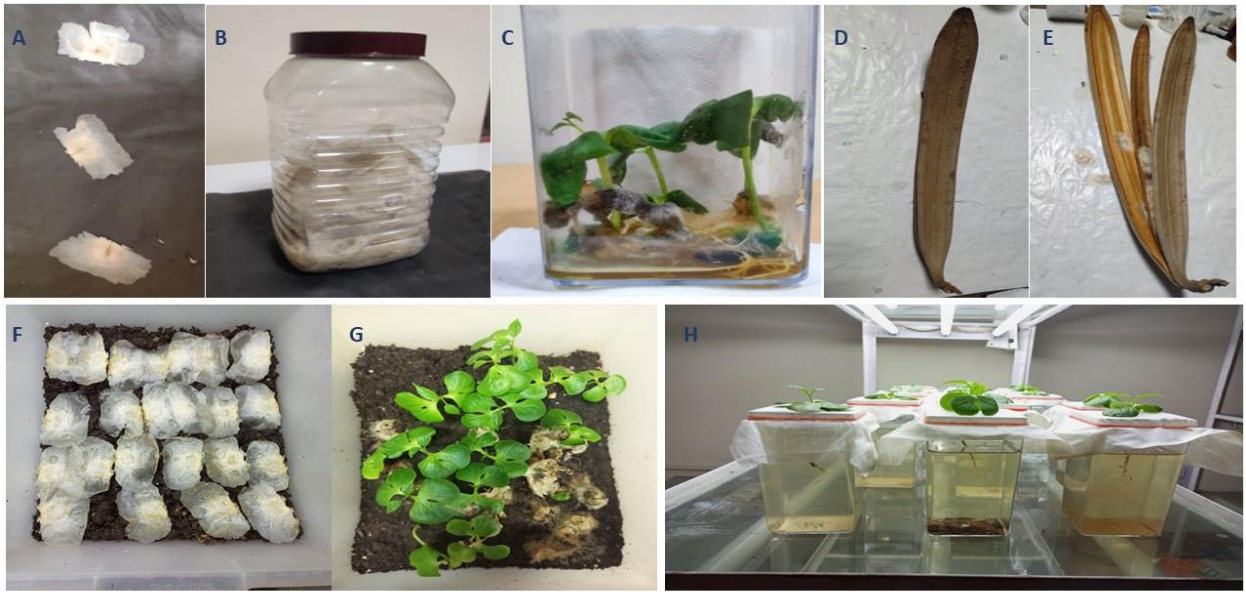

Figure 1: (A) represents the seeds of *O. indicum*; (B) represents the bottle were the seeds were stored; (C) represents the germination of the seeds after gibberellic acid; (D) and (E) represents the seed pods closed and opened up; (F) represents the seeds kept in soil for its germination and after germination (G) the plants could be used for hydroponics (H).

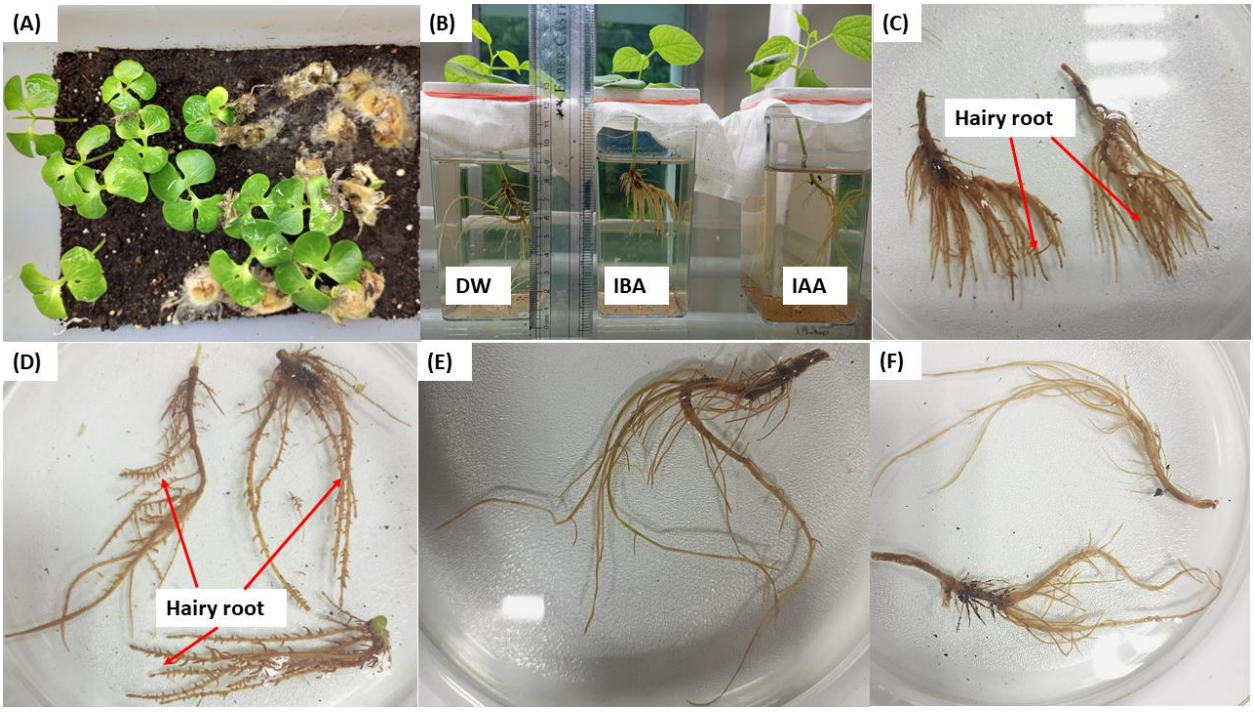

Figure 2: Showing hairy root generation of *O. indicum* plant due to IAA and IBA treatment, (A) Germination in soil from HiGrow soil after 60 days under light 3000 lux, (light hour 12h / dark12h) temperature (28+/-3 in light and 22+/-3 degree celcius in dark); (B) transferred plant from (A) to hydroponic system with mineral solution prepared with 10 g HiGrow soil in 100 ml sterile distilled water and subsequently supplimented with IBA and IAA after every 6 days days (5 mg/L) (C) &

(D) profound hairy root generated due to IBA (5mg/L) after 60 days. (E) showing elongated primary root due to only treatment with IAA (5mg/L) and (F) without any hormonal treatment.

Objective 2: Compound purification

2.1. Isolation of baicalein and chrysin

The methanolic extract of powdered root bark (40.0g/400 ml of MeOH through percolation method) was prepared and column chromatography was carried out with benzene and chloroform to isolate baicalein and chrysin respectively (Dinda *et. al.*, 2007). The benzene fraction was further purified with petroleum benzine: benzene (3:7) followed by petroleum benzine: ethyl acetate (4:1) for pure baicalein. The chloroform fraction was further purified with petroleum benzine: chloroform (1:4) to get chrysin. The plant samples used for the compound purification were collected from Arunachal Pradesh (I) (root bark (R)) and Tezpur (II) (Stem bark(s)). The sample name for purified baicalein (b) are IIs1b, IR1b, IR*1b and for purified chrysin (c) fraction the sample name were IIs1c, IR1c, IR*1c.

2.2. Thin layer chromatography (TLC) analysis

Confirmatory TLC analysis of compounds are carried out following method described by by kang *et al.* 2019 for baicalein, Seetharaman *et al.* 2016 for chrysin. The TLC analysis was conducted by using TLC silica gel 60 F_{254} (Merck KgaA, Darmstadt, Germany). The samples were loaded in the TLC plates, dried and then developed in chloroform: methanol (20:1 v/v), toluene: ethyl acetate: acetic acid (36:12:5 v/v) for baicalein and chrysin respectively. Pure compounds of Baicalein, chrysin were used as standard. All the TLC plates were then observed under UV light and also stained with iodine beads.

The rf value for baicalein, IIs1b, IR1b and IR*1b are 0.79, 0.78, 0.79, 0.769 (See figure 2, A1 and A2) respectively and for chrysin, IIs1c, IR1c and IR*1c 0.717, 0.692, 0.692 and 0.705 (See figure 2, B1 and B2) respectively.

2.3. FTIR analysis

The FTIR analysis of the pure compounds and the purified compounds was done by using the FTIR spectrophotometer (IMPACT 410, NICOLET, USA). For Baicalein, the peaks which were similar for pure compound and extract (I1b) were 3445, 2997, 2917, 2852, 2000, 1646, 1435, 1311, 1021 cm-1, except 2264 cm-1 which was unique to the standard but was not found in I1b (Fig 2, D). For chrysin and I2c the similar peaks were 3440, 2996, 2851, 1640, 1436, 1311, 1048, 955, 709 cm-1 (Fig 2, E). For Oroxylin A and IRHEE the similar peaks were 3445, 2919, 2852, 1317, 1650, 1019 cm-1 (Fig 2, F).

2.4. Quantification using UV-Visible spectroscopy

For the quantification of baicalein, chrysin and oroxylin A UV-visible spectroscopy (Split Beam UV-visible spectrophotometer, Genesys 50, Thermo Scientific) was used. A standard curve of baicalein and chrysin was prepared with concentration of 12.5, 1.25 and 0.125 µg/ml and for oroxylin A the concentration was 6.25, 0.625, 0.0625 µg/ml. Baicalein, chrysin and oroxylin A gave a maximum absorbance at 280, 270 and 274 nm respectively. The standard curve was used to identify concentration of the compounds in the extracted samples. The amount of compound calculated is shown below:

Sample Name	IIS1B	IR1B	IR*1B	IIS2C	IR2C	IR*2C	IRHEE
Amount of compound per 1 mg of crude extract (µg)	67.785	21.327	4.440	105.771	38.227	26.298	127.389

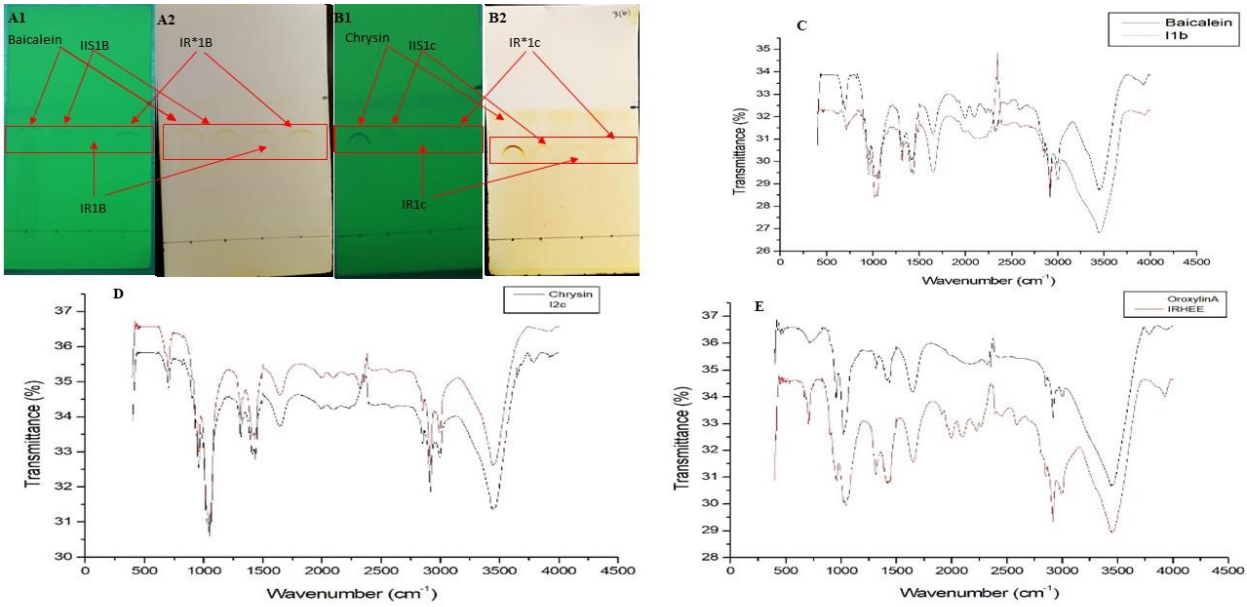

Fig 2: (A1, A2) Represents the TLC plate of pure baicalein and purified baicalein sample from different locations; (B1, B2) Represents the TLC plate of pure chrysin and purified chrysin sample from different locations; (C) FTIR report of baicalein and purified baicalein ; (C) FTIR report of pure and purified chrysin ; (C) FTIR report of pure and purified Oroxylin A.

Objective 3: Bioactivity

3.1. Antioxidant activity

DPPH stable free radical scavenging capacity was estimated according to Brand-Williams et al., (1995) and Shyu and Hwang (2002) with slight modifications. In the assay, 1.0 mL of diluted extract was mixed with 2.0 mL of 0.10 mM solution of DPPH in methanol. The mixture was incubated in the dark at room temperature for 30 min, and the absorbance at 517 nm was measured. All tests were performed in triplicate. (Figure 3, A).

Calculation of DPPH scavenging activity = (1-Abs of sample/Abs of control) X 100

The antioxidant activity was shown by pure baicalein (96.87%), IIS2c (12.87%), IR*2C (2.34 %) and IRHEE (73.73%), whereas the other samples did not show any antioxidant activity.

3.2. Antibacterial activity

For antimicrobial assay Radhika *et al*. (2011) was followed with slight modification. Extracts of root and stem of *Oroxylum indicum* were taken and agar well diffusion method was used. The medium used for antibacterial testing was Muller Hinton agar (MHA) (Himedia). The extracts were dissolved in DMSO (Dimethyl sulphoxide, Merck). In the prepared wells 100 µl of test solution is added to the wells on the MHA plate. Gentamicin (1 mg/ml) for both gram negative and positive bacteria were used with incubation at 37°C overnight. The diameter of the zone of inhibition was measured (in mm). The antibiotic showed a high zone of inhibition (See Figure 3: D, E, F).

Table 1: Showing antibacterial activity of the pure and purified compounds.

Sl No.	Name of the extract	Zone of inhibition (mm)			
		Staphylococcus aureus MTCC 3160	*Bacillus cereus* MTCC 430	*Klebsiella pneumoniae* MTCC 618	*Escherichia coli* MTCC 40
1	Baicalein	14	17	14	17
2	Chrysin	ND	ND	ND	ND
3	Oroxylin A	ND	18	ND	ND
4	IIS1b	ND	14	ND	ND
5	IR1b	ND	22	ND	ND
6	IR*1b	ND	22	ND	ND
7	IIS2c	14	ND	15	ND
8	IR2C	ND	ND	ND	ND
9	IR*2C	ND	ND	ND	ND
10	IRHEE	ND	16	ND	ND

I- Arunachal pradesh sample, II- Tezpur sample, S- stem bark powder, S*- stem bark coarsed, 1- benzene fraction, 2- chloroform fraction, b- baicalein, c- chrysin, IRHEE- arunachal pradesh root bark sample Hexane-Ethyl acetate- ethanol extract, ND- Not Detected.

3.3. Anticancer activity

For performing the MTT assay work done by Masoomi *et al.* (2017) was followed with slight modification. Briefly, 10,000 cells (MDA-MB-231 breast cancer cell line) were seeded in 96 well plates using 10% FBS containing DMEM and were incubated for 12 hours. On the day of the experiment different concentrations (0, 10, 25, 50, 100, 200, 300, 400, 500 µM) of each three pure compounds (baicalein, chrysin, oroxylin A) and for fractionated samples the concentration were 0, 12.5, 25, 50, 100, 200, 300 µg/ ml were added in triplicates and cells were allowed to be incubated at 37 degrees C and 5 % CO2 incubator. Three hours before the completion of 24 hours 15µl of MTT (5mg/ml of PBS) was added to each well and cells were incubated for three hours. At the end of 24 hours, media was discarded carefully using a pipette, and cells were lysed using MTT lysis solution (MTT lysis solution : 11g SDS, 0.2M HCL, 50% Isopropanol). Cells were covered in aluminum foil and were incubated for 15 min at room temp and spectrophotometric O.D. was measured at 595 nm.

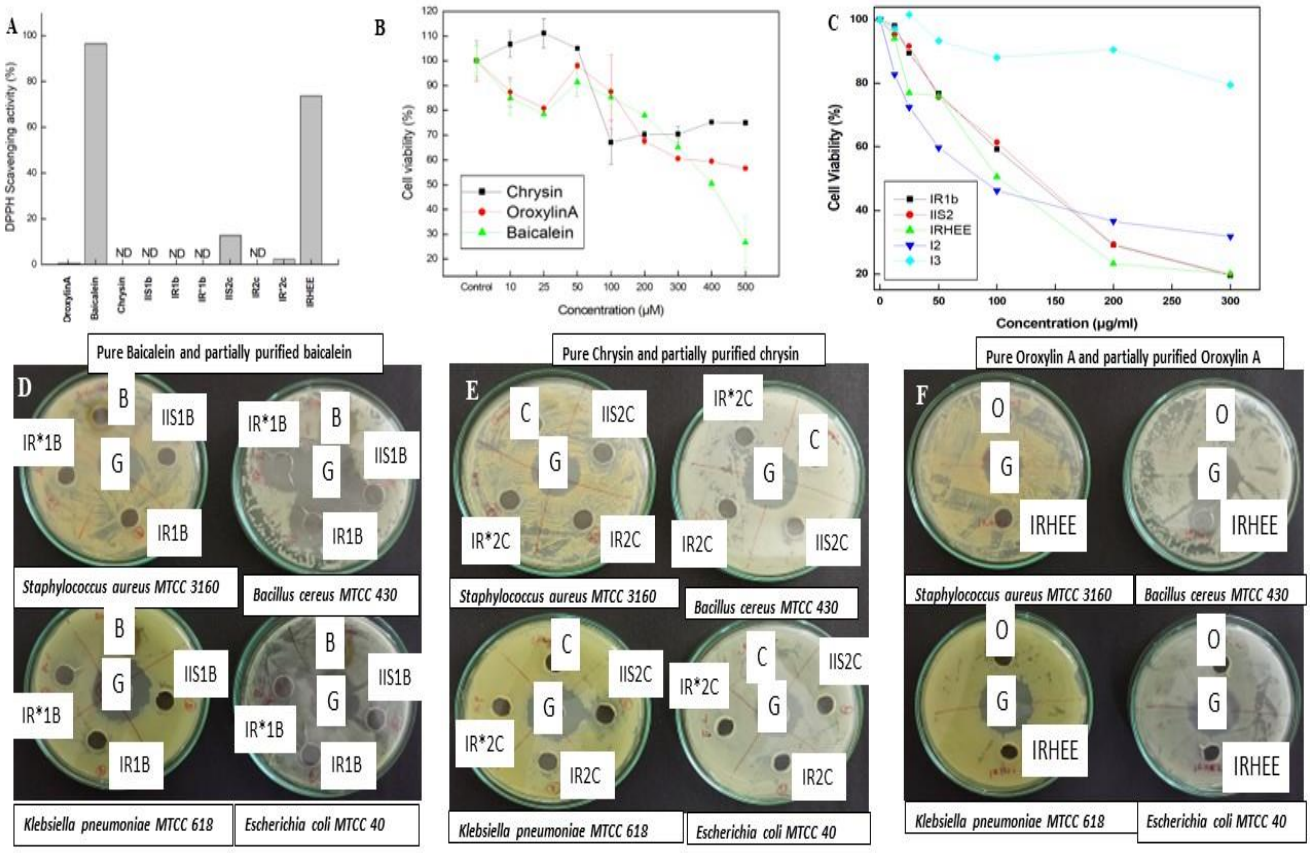

Figure 3 (A) Showing the DPPH activity of pure compounds along with the partially purified compounds. (B) MTT assay data against breast cancer cell line (MDA-MB-231) for pure compounds of Baicalein, chrysin and Oroxylin A; (C) Showing the MTT assay data against breast cancer cell line (MDA-MB-231) for partially purified compound baicalein (IR1*b) , chrysin (IIS2c), partially purified Oroxylin A (IRHEE) and chloroform fraction (I2); (D, E, F) representing the antibacterial activity of the pure compounds along with the partially purified compound against S. aureus MTCC 3160, Bacillus cereus MTCC 430, Klebsiella pneumoniae MTCC 618, Escherichia coli MTCC 40.

I- Arunachal pradesh sample, II- Tezpur sample, S- stem bark powder, S*- stem bark coarsed, 1- benzene fraction, 2- chloroform fraction, b- baicalein, c- chrysin, IRHEE- arunachal pradesh root bark sample Hexane-Ethyl acetate- ethanol extract.

The results have shown that for pure compounds of baicalein, Chrysin and Oroxylin A the anticancer activity is not much significant till the concentration of 100 µM concentration (reported in 2021). As the concentration of the concompounds were increased all the three compounds have shown a significant decrease in cell viability where baicalein has shown a maximum reduction in cell viability of 26.6 %, followed by oroxylin A with 56.68% and chrysin showing 74.95% cell viability at a concentration of 500 µM (Figure 3, B).

The purified baicalein (IR1*b) , chrysin (IIS2c), partially purified oroxylin A and chloroform fraction (I2) have shown decrease in cell viability with increase in concentration from 12.5 µg/ml to 300 µg/ml. Though the butanol fraction (I3) has shown less anticancer activity even at the highest dose of 300 µg/ml with cell viability of 79.53% (Figure 3, C).

3.4. Growing *O. indicum* in vitro in hydroponics and agar medium: Germination of high value threatened medicinal plant *O. indicum* is reported to be difficult (Sing *et al.*, 2014). Germination was carried out with previously standardized (mentioned in details in 2020-21 report) methods with slight modifications. During imbibition instead of pure distilled water gibberellic acid solution (1 mM). It has been observed that seeds lose their germination completely within a period of year, if stored in airtight plastic jar or packet after removal from seed pod. During the germination experiment we have seen that only seeds stored with the intact pod are viable even after one year of storage in room temperature (Figure 1, C). The seeds of the *O. indicum* in a plastic jar and with pods are presented in Figure 1(A,B,D,E) and Figure 2 (A-F).

Germination of the *O. indicum* seeds were carried out in a plastic container with soil (Growpur, potting mix soil) (Figure 1, E). The seeds were allowed to germinate at temperature of 26℃ ± 2℃ with a photoperiod of 10 hours and with light intensity of (3000 lux). The seeds germinated within a week and after two weeks the plant reached 2 leaf stage (Figure 1, F). As the plant reached 4 leaf stage, the plant was used for hydroponics cultivation which then was treated with rooting hormone Indole-3-acetic acid (IAA) (5 mg/L) and Indole-3- butyric acid (IBA) (5 mg/L) for hairy root formation. This experiment was done so that there will be sufficient samples of hairy roots of *O. indicum* for compound purification (see Figure 1, G).

3.5. Compound purification from *O. indicum* seed grown using hydroponics as well as nutrient agar medium (i.e., *in vitro*):

3.5.1 Preparation of extracts:

The samples were dried overnight in a hot air oven at 60^{o} Celsius. The samples were weighed and recorded respectively. Using a mortar and pestle, the dried leaf and root samples were ground into a coarse powder. Each powdered plant material was then overnight dissolved in 20 ml of 100% methanol in falcon tubes and labeled as follows:

Table 1: Different plant samples	
Samples	**Labeling**
IBA treated roots	IBA
IAA treated roots	IAA
Distill water treated roots	DW
R. rhizozene root	RRR
GA3 treated roots	GA3
Control	C
Leave sample 1	RRL1
Leave sample 2	RRL2

The methanolic solvent containing the plant extract was transferred to a separate vessel, and the plant sample was resuspended in 20 ml methanol. The procedure was subsequently repeated two more times. And the extract is concentrated by being evaporated to dryness in a hot air oven at 40^{o}C.

3.5.2 High performance liquid chromatography (HPLC) analysis :

3.5.2.1 Sample preparation: Stocks of each extracts was prepared by dissolving the concentrated extracts in 8ml of methanol: miliQ H_2O (1:1 ratio) and filtered using 0.2 (μm) micron syringe filter (PTFE). This stock was then diluted tenfold by dissolving 100 μl of filtered sample in 900 μl of methanol+ miliQH2O (1:1 ratio).

3.5.2.2 HPLC condition:

The samples were then analyzed in HPLC according to two protocols mentioned by Li et al., 2005 and Song et al., 2018 with slight modification.

According to Li et al., 2005 the mobile phase A used was methanol containing 2% acetic acid and Mobile phase B was MiliQH_2O.The gradient protocol performed is as follows: 0-3 minutes, 0-20% A; 3-10 minutes, 20-50% A; 10-20 minutes, 50-50 % A; 20-35 minutes, 50-80%; 35-45 minutes, 80-80% A; 45-50minutes, 80-100% A; 50-55 minutes, 100-0% A. The flow rate was set at 0.7ml/min and detector under 280nm. The volume injected was 20ul for every sample.

According to Song et al.,2018 the mobile phase A used was methanol containing 2% acetic acid and Mobile phase B was MiliQH_2O.The gradient protocol performed is as follows: : 0-15minutes, 25-55% A; 15-18minutes, 55-70 % A; 18-26 minutes, 70-70 %A; 26-28minutes, 70-25 % A; 5 minutes, 100-0% A. The flow rate was set at 0.7ml/min and detector under 280nm. The volume injected was 20ul for every sample.

The eluent collected from HPLC was further analyzed by Fourier Transformed Infrared Spectroscopy and Mass spectroscopy.

3.5.3 Fourier transformed infrared spectroscopy (FTIR) analysis:

The samples were subjected to Fourier transformed infrared spectra (FTIR). FTIR analysis was done from wave numbers 400cm^{-1} to 4000cm^{-1}. The FTIR result was recorded by IMPACT 410, NICOLET, USA FTIR spectrophotometer with Software: OMNIC E.S.P.5.0. Measurements were taken at a resolution of 1 cm^{-1}. This was carried out in the Dept. of Chemical Sciences, Tezpur University. The analysis was done using ORIGIN software.

3.5.4 Liquid chromatography mass spectroscopy (LC MS) analysis:

The LCMS analysis was carried out on an Agilent 6100, Germany using a C18 reverse phase column (2.1X 50 mm, 1.8 micro m). For 15 minutes, the sample was eluted in an isocratic manner with LCMS-grade water and methanol at a 50:50 ratio. The water contains 0.1% formic acid and 0.1% ammonium format. With a flow rate of 0.2 ml/minute, a sample volume of 5 L was injected. Ionisation was carried out in the mass range 100-1000 m/z using positive ESI mode. MestReNova software v14.1.1-24571 was used to examine the LCMS results.

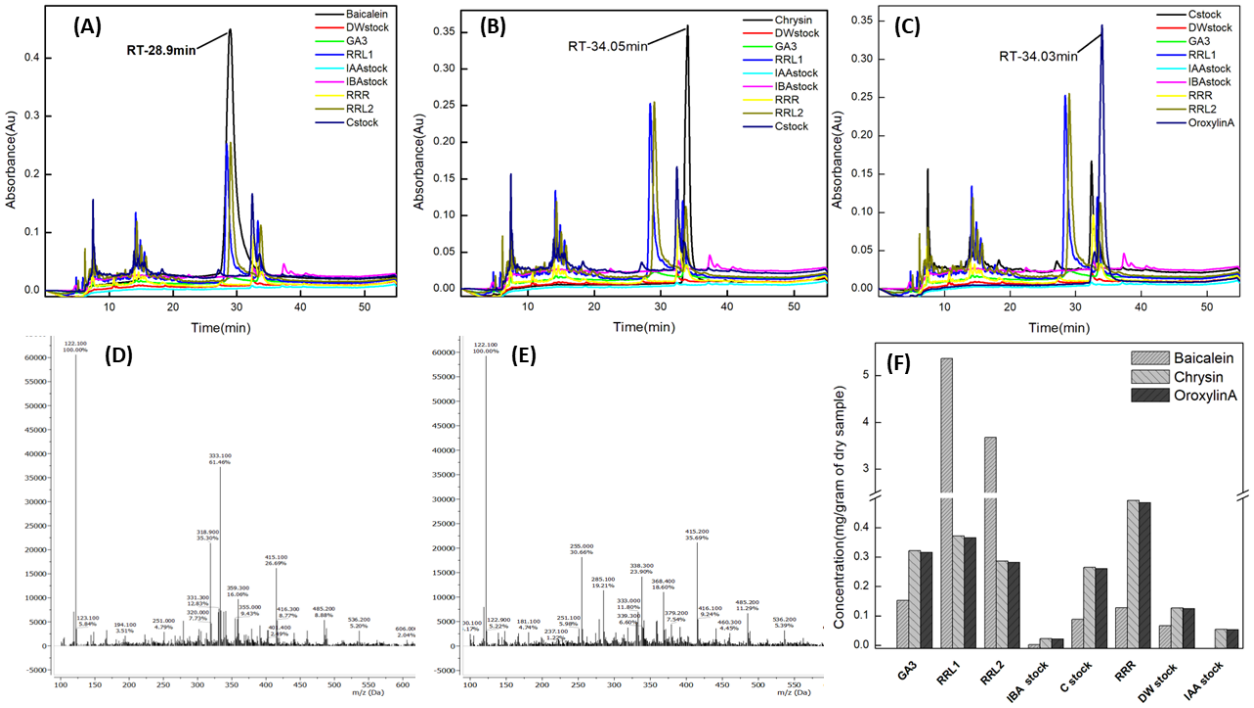

Figure 1: HPLC profile of (A) baecalin, (B) chrysin, (C) oroxylin A, (D) liquid chromatography-mass spectrometry (LC-MS) analysis of the HPLC peak collected at retention period 28 minutes. It is observed that there is an ammonium ion adduct (288 m/z) with the molecular mass of the baicalein, (E) liquid chromatography-mass spectrometry (LC-MS) analysis of the HPLC peak collected at retention time of 34 minutes shows m/z values of 255.000 (100%) (M+H+) and 285.000 (71.08%) (M+H+), which indicates chrysin and oroxylin A, respectively, (F) concentration of baicalein, chrysin, oroxylin A in each dry sample showing that the concentration

of baicalein is maximum in RRL1 and RRL2 sample, whereas concentration of chrysin and oroxylin A in RRR sample.

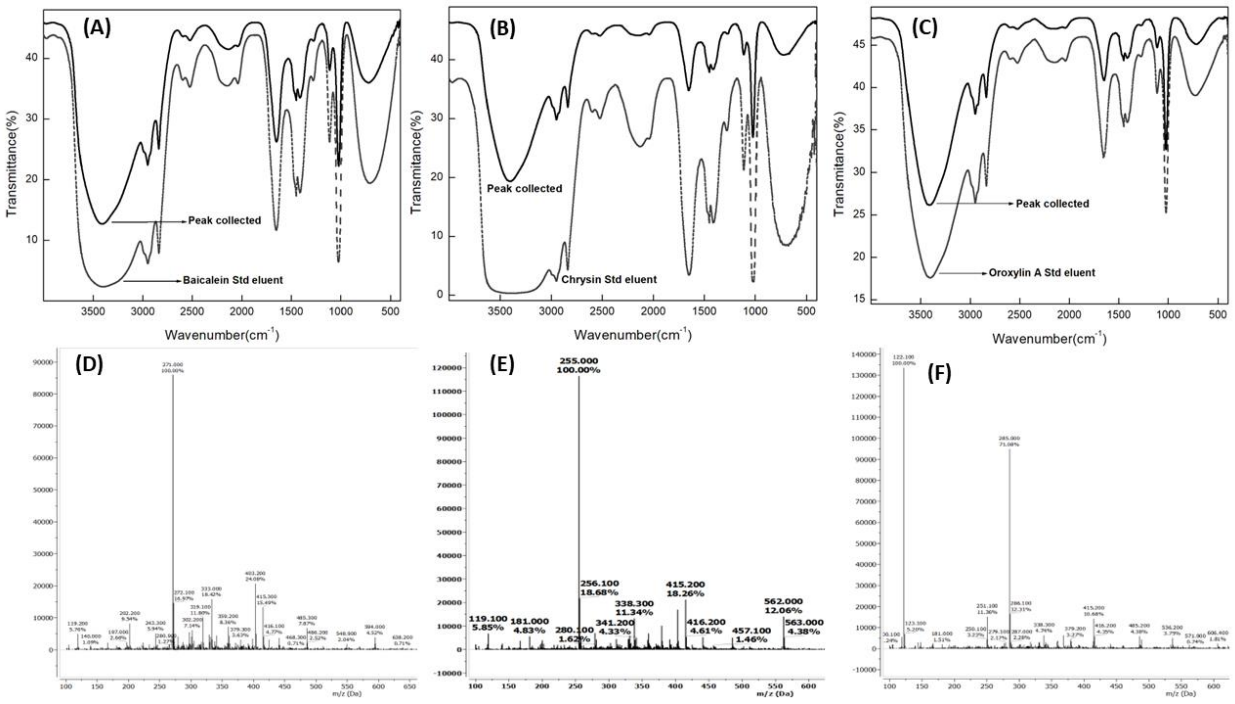

Figure 2: Showing FTIR data for (A) baicalein; (B) chrysin; and (C) oroxylin A and MS data for (D) Liquid chromatography-mass spectrometry (LC-MS) analysis of pure baicalein showing significant peak at m/z 271.000 with a 100% abundance indicating H+ adduct to its compound molecular weight; (E) Liquid chromatography-mass spectrometry (LC-MS) analysis of pure Chrysin showing significant peak at m/z 255.000 with a 100% abundance indicating H+ adduct to its compound molecular weight; (F) Liquid chromatography-mass spectrometry (LC-MS) analysis of pure Oroxylin A showing significant peak at m/z 285.000 with a 71.08% abundance indicating H+ adduct to its compound molecular weight.

B2. Summary and Conclusions of the Progress made so far:

Anticancer activity is analyzed with standard compounds (with maximum efficacy of 73.4, 30 and 45 % viability loss with baicalein, chrysin and oroxylin A respectively during treatment with 500 microM). The purified compound baicalin 135.12 micro g/ml (500microM) which show 73.4% viability loss in cancer cells compared to IR1b (purified baicalin fraction) at 300 micro g/ml provides comparable anticancer activity (80.47 % viability loos). Similarly pure compound chrysin 127.12 micro g/ml (500 micro M) decreases viability of cancer cells by 25.05% compared to purified fraction IIS2c (purified chrysin fraction) 300 micro g/ml decreases viability of cancer cells by 80.05%. FTIR analysis of the repurified chrysin fraction I2c does not show indication of impurity. Further research may be needed to validate the anticancer activity. Oroxylin A (pure

compound) 500 micro M (142.13 micro g/ml) decreases viability of cancer cells by 43.32 %, compared to purified fraction IRHEE (purified fraction for Oroxylin A) decreases viability of cancer cells by 79.91% at concentration 300 micro g/ml.

The antioxidant activity was shown by pure baicalein (96.87%), IIS2c (12.87%), IR*2C (2.34 %) and IRHEE (73.73%), whereas chrysin, oroxylin A and other purified compounds did not show DPPH scavenging activity.

Pure baicalein has shown antibacterial activity against all the four bacterials species i.e., *Staphylococcus aureus* MTCC 3160, *Bacillus cereus* MTCC 460, *Klebsiella pneumoniae* MTCC 618 and *Escherichia coli* MTCC 40 with zone of inhibition of 14, 17, 14, 17 mm respectively. Whereas, oroxylin A, IIS1b, IRSb, IR*1b and IRHEE has shown antibacterial activity against Bacillus cereus MTCC 430 with zones of inhibition of 18, 14, 22, 22 and 16 mm respectively. IIS2c has shown antioxidant activity against *Staphylococcus aureus* MTCC 3160 and *Klebsiella pneumoniae* MTCC 618.

The amount of baicalein present in per gram of dry extract is as follows; RRL1 > RRL2 >DW> RRR > IAA > GA3 > C > IBA. The amount of chrysin present in per gram of dry methanolic extract is as follows; RRL2> RRL1 >RRR>DW> GA3 > C> IAA> IBA. The amount of oroxylin A present in per gram of dry methanolic extract is as follows; RRL2 > RRL1> RRR > DW > GA3 > C > IAA > IBA.

From the HPLC chromatograms it was observed that under Li et al.'s 2005 methodology the baicalein standard gave its maximum peak at retention time 28.99 min, whereas chrysin and oroxylin A standards produced their maximum peaks at retention times 34.05 min and 34.03 min, respectively. Oroxylin A and chrysin have identical retention times, which may be explained by how closely their structures resemble one another. Except for the IAA-treated sample, all of the samples showed two main peaks with retention times of roughly 28 and 34 minutes, which were the peaks of standard chrysin and baicalein, respectively.

Presence of bioactive compounds baicalein, chrysin, oroxylin A in the extracts of *O.indicum* was confirmed through FTIR analysis of the eluents collected from HPLC. From the FTIR spectra of it is observed that the pure baicalein standard exhibits characteristic major absorption peaks at 3450 cm-1, 2951 cm-1, 2840 cm-1, 1645 cm-1, 1452 cm-1 and 1020 cm-1 respectively. O-H stretching is indicated by the absorption band at 3450 cm-1, while C-H stretching is indicated by the bands at 2951 cm-1 and 2840 cm-1. The carbonyl group is indicated by the peak at 1645 cm-1, while the C=O group is represented by the peak at 1027 cm-1. Additionally, the stretching vibration of the C=C (benzene ring) occurs at 1452 cm-1. The collected eluent's FTIR spectra at a retention time of 28.9 min show characteristic absorption bands with similar widths to the standard baicalein at 3419 cm-1 (O-H stretch), 2952 cm-1 (C-H bond vibration), 2833 cm-1 (C-H bond vibration), 1648 cm-1 (C=O bond vibration), 1446 cm-1 (C=C bond vibration), and 1020 cm-1

(C=O bond vibration). As a result, it was found that the peak obtained at 28.9 minutes of retention time and the infrared spectra characteristic region (4000-400 cm^{-1}) of Standard Baicalein eluent displayed similar patterns suggesting that the peak collected is also baicalein.

B3. Details of New Leads Obtained, if any: Seed of the *O. indicum* may be germinated (poor germination of the seed under natural condition is one of the reason behind *O. indicum* becoming endangered species) if imbibed with distilled water at proper temperature, light hour and duration. We found germination of *O. indicum* seed can further enhance (early germination) by imbibition in gibberellic acid (1mM) solution. It has been observed that seed stored with the seed pod are more viable compared to one which is removed before storing. The stem of the plant is found to have a hollow canal in the center of the stem with 1/4th of its diameter. Anticancer activity of the purified compound (MDA-MB-231 breast cancer cell line) is found to be comparable to that of pure compounds. The FTIR (liquid sample) of DMSO dissolved pure compounds (Oroxylin A, baicalin and chrysin) may be the first report as literature on this is very scanty. FTIR of the pure compounds found to have minor differences with one that is purified from *O. indicum* root bark. Some of the anticancer compounds are found to be lacking antibacterial and antioxidant activity. From all of the results, it could be summarized that the extract revealed the presence of medicinally important flavonoid contents baicalein, chrysin and oroxylin a by spectroscopic techniques. The extracts from leaves and *Rhizobium rhizogenes* infected mediated leaves and hairy roots had high contents baicalein, chrysin and oroxylin A.

B4. Details of Publications, technology developed & Patents, if any emanated from the project: One manuscript incorporating all information is under communication.

B5. Benefits gained through Twinning: The manpower got skilled in laboratory techniques like fractionation, column chromatography, TLC and HPLC during purification of baicalein, chrysin and oroxylin A, from *O. indicum* root bark. Manpower also got skilled in optimizing seed germination of endangered plant *O. indicum*, which are difficult to germinate under natural conditions. Manpower is getting acquainted with anticancer activity assay design and troubleshooting interference of plant compounds during MTT assay. Additionally manpower got experience in hydroponic cultivation of *O. indicum*.

Section-C: Details of Grant Utilization#

C1. Equipment Acquired or Placed Order with Actual Cost: Equipment purchase is over in the last financial year. Not any.

C2. Manpower Staffing and Expenditure Details: Rs. 6,99,293/- (FY 2019-23).

First JRF resigned on dated 18/02/2021, a Project Assistant appointed for 3 months to prevent the ongoing project work from halt (tenure ends on 19/06/2021) during complete lockdown and prevention notice by authority for project staff to visit the laboratory. Waiting for DBT approval and redesignation of JRF/SRF position to Project Associate I/II (do not need to have NET/GATE as per DST rules) with the same financial sanction and then only to start a new manpower recruitment process by TU. After receiving funds for FY 2021-22 on 18/10/2021 Project assistant is appointed from 01/11/2021 to 01/09/2022 following Tezpur University procedure.

C3. Details of Recurring Expenditure:Rs. 13,41,268/-

C4. Financial Requirements for the Next Year with Justifications: Not any.

#Grant utilization details (UC & SC, Assets Certificate & manpower details are also with this report for FY 2019-23. Bharat kosh receipt and uploading of UC & SE in PFMS is requested to the Finance Section, Tezpur University and will be submitted once received).

(J. P. Saikia)

[Signature(s) of the Investigator(s)]

Instructions:

(i) All the information needs to be provided; otherwise the Progress Report will be treated as incomplete. In case of 'Nil' / 'Not Applicable' information, the same may be indicated.

(ii) In case of multicentre project, a combined Progress Report should be submitted incorporating the progress of all components. The Project Co-coordinator/ PI will be responsible for this.

*(iii) *Please indicate the reporting period [i.e. Year 1/2/3/4/5].*

(iv) Submission of Progress Report by the end of the 11^{th} month of grant sanction is linked with further continuation of the project and timely release of funds for the next year.

Appendix-B

Utilisation Certificate

(For the financial year ending 1st April 2022 to 31st March 2023)

(Rs. in Lakhs)

1. Title of the Project/Scheme: **Enhanced Production of anticancer flavones- Baicalein, Chrysine and Oroxylin A, from *Oroxylum indicum* tissue culture and its studies on its chemopreventive activities.**
2. Name of the Organization: **Tezpur University, Napaam-784028, Assam, India**
3. Principal Investigator: **Dr. Jyoti Prasad Saikia, Assistant Professor, Department of Molecular Biology and Biotechnology, Tezpur University, PO-Napaam, Tezpur- 784028, Sonitpur, Assam, India**
4. Dept. of Biotechnology sanction order No. & date of sanctioning the project: **BT/PR25000/NER/95/950/2017, Dated: 02/09/2019**
5. Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given: **Rs.2.65656/- (BT/PR25000/NER/95/950/2017, Dated: 23/09/2021).**
6. Amount received from DBT during the financial year **(BT/PR25000/NER/95/950/2017, Dated: 23/09/2021): Nil**
7. Other receipts/interest earned, if any, on the DBT grants: **Rs.0.02761/-**
8. Total amount that was available for expenditure during the financial year (Sl. Nos. 5, 6 and 7): **Rs.2.68417/-**
9. Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed): **Rs.**1.11057/- **(excluding refund)** including interest.
10. Unspent balance refunded, if any *(Please give details of cheque No. etc.):* **Rs.1.57360/-**
11. Balance amount available at the end of the financial year: Total balance amount available at the end of the year is **Nil.**
12. Amount allowed to be carried forward to the next financial year vide letter No. & date: **None**

1. Certified that the amount of **Rs.1,11,057/-** (Rupees One Lakhs Eleven Thousand Fifty Seven Only) mentioned against col. 9 has been utilized on the project/scheme for the purpose for which it was sanctioned and that the balance of **Nil** remaining unutilized at the end of the year has been surrendered to Govt.
2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned. Kinds of checks exercised:1. (Cash Book), 2.(Ledgers), 3.(Vouchers), 4.(Bank Statements), 5.

24/5/23

(Signature of PROJECT INVESTIGATOR with Stamp)

(J. P. Saikia)
Assistant Professor,
Dept. of Molecular Biology & Biotechnology
Tezpur University
Napaam, Tezpur- 784028

(Signature of FINANCE OFFICER with Stamp)

Finance Officer
Tezpur University

24/5/23

(Signature of HEAD OF THE INSTITUTE with Stamp)

Registrar
Tezpur University

(To be countersigned by the DBT Officer-in-charge)

Appendix-C

Statement of Expenditure referred to in para 9 of the Utilisation Certificate

Showing grants received the Department of Biotechnology and the expenditure incurred during the period from 1st April 2022 to 31st March 2023. (Rs. in lakhs)

Item	Unspent Carried forward from Previous year	Grants received from DBT during the year	Other receipts/interests earned, if any, on the DBT grants	Total of Col. 2+3+4	Expenditure (excluding commitments) incurred during in year	Balance (5-6)	Remarks
1	2	3	4	5	6	7	8
1. Non-Recurring							
(i) Equipments	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	Done
2. Recurring							
(i) Human Resource	1.90503	0.00000	0.00000	1.90503	1.00000	0.90503	
(ii) Consumables	0.12943	0.00000	0.00000	0.12943	0.00000	0.12943	
(iii) Travel	0.50000	0.00000	0.00000	0.50000	0.00000	0.50000	
(iv) Contingency	0.03914	0.00000	0.00000	0.03914	0.00000	0.03914	
(v) Overheads	0.05016	0.00000	0.00000	0.05016	0.05016	0.00000	
(vi) Interest Earned	0.03280	0.00000	0.02761	0.06041	0.06041	0.00000	
(vii) Refund	0.00000	0.00000	0.00000	0.00000	1.57360	-1.57360	
Total	**2.65656**	**0.00000**	**0.02761**	**2.68417**	**2.68417**	**0.00000**	

Balance: Nil

24/5/23

(Signature of PROJECT INVESTIGATOR with Stamp)

(J. P. Sarkar)
Assistant Professor,
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Tezpur University
Napaam, Tezpur- 784028

(Signature of FINANCE OFFICER with Stamp)

Finance Officer
Tezpur University

26/5/23

(Signature of HEAD OF THE INSTITUTE with Stamp)

Registrar
Tezpur University

FINALCONSOLIDATED STATEMENT OF EXPENDITURE
(FOR FINAL SETTLEMENT OF ACCOUNTS)

1. Title of the Project : Enhanced production of anticancer flavones- Baicalein, Chrysine and Oroxylin A, from Oroxylum indicum tissue culture and studies on its chemopreventive activities.
2. Sanctioned Project Cost : Rs.26,07,192/- for Tezpur University Part of total Rs.72,54,384/-
3. Revised cost, if any : Nil.
4. Duration of the project : Three (03) years (Extended up to March 2023).
5. Sanction Order No. & Date :BT/PR25000/NER/95/950/2017 Dated 02/09/2019.
6. Date of commencement of Project : September 02nd 2019.
7. Extension, if any : No cost extension from September 2022 to March 2023.
8. Date of completion of project : March 31st 2023.

Details of grant, expenditure and balance (Rs. in Lakh)

S. No.	Heads	Sanctioned Cost	Year-wise Releases made					Year-wise Expenditure incurred					
			1st yr	2nd yr	3rd Yr	4th Yr	Total	1st yr	2nd yr	3rd yr	4th yr	Total	Balance
A.	**Non-recurring**	pageno.-											
	Equipments	5.00000	5.00000	0.00000	(-) 3.34000	0.00000	1.66000	0	1.66000	0.00000	0.00000	1.66000	0.00000 (adjusted)
B.	**Recurring**												
1.	Manpower	12.57192	4.01796	0.00000	3.88000	0.00000	7.89796	1.09000	2.79000	2.11293	1.00000	6.99293	0.90503
2.	Consumables	4.00000	1.500000	0.00000	1.43870	0.00000	2.93870	0.00000	1.43870	1.37057	0.00000	2.80927	0.12943
3.	Travel	1.50000	0.50000	0.00000	0.05035	0.00000	0.55035	0.05035	0.00000	0.00000	0.00000	0.05035	0.50000
4.	Contingency	1.50000	0.50000	0.00000	0.50000	0.00000	1.00000	0.03500	0.49803	0.42783	0.00000	0.96086	0.03914
5.	Overhead	1.50000	0.50000	0.00000	0.43927	0.00000	0.93927	0.0000	0.43927	0.44984	0.05016	0.93927	0.00000
	Total	21.07192	7.01796	0.00000	6.30832	0.00000	13.32628	1.17535	5.16600	4.36117	1.05016	11.75268	1.57360
	Grand Total (A+B)	26.07192	12.01796	0.00000	2.96832	0.00000	14.98628	1.17535	6.82600	4.36117	1.05016	13.41268	1.57360

(PROJECT INVESTIGATOR)
Assistant Professor,
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Napaam, Tezpur-784028

(HEAD OF THE INSTITUTE)
Registrar
Tezpur University

(FINANCE OFFICER)
Finance Officer
Tezpur University

Appendix-A

Details of Assets acquired wholly or substantially out of Govt. grants Register to be maintained by Grantee Institution

1. Name of the Sanctioning Authority: DBT
2. Name of the Grantee Institution: Tezpur University
3. No. & Date of sanction order: BT/PR25000/NER/95/950/2017, Dated: 02/09/2019
4. Amount of the sanctioned grant: Rs.26,07,192/- for Tezpur University Part of total Rs.72,54,384/-
5. Brief purpose of the grant : Enhanced production of anticancer flavones- Baicalein, Chrysine and Oroxylin A, from Oroxylum indicum tissue culture and studies on its chemopreventive activities.
6. Whether any condition regarding the right of ownership of Govt. in the Property or other assets acquired out of the grant was incorporated in the grant-in-aid sanction order. None
- *7. Particulars of assets actually credited or acquired. : ELISA Reader (96 well plate reader)
8. Value of the assets as on31/3/2021:Rs.1,66,000/- (Rupees one lakh sixty six thousand only)
9. Purpose for which utilised at present: For "Enhanced production of anticancer flavones- Baicalein, Chrysine and Oroxylin A, from Oroxylum indicum tissue culture and studies on its chemopreventive activities."
10. Encumbered or not : not
11. Reasons, if encumbered : not arise
12. Disposed of or not : Not
13. Reasons and authority, if any, for: Disposal Not arise
14. Amount realised on disposal : Not arise
15. Remarks: In Working condition

18/8/23

(PROJECT INVESTIGATOR with Stamp)

(J. P. Saikia)
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(FINANCE OFFICER with Stamp)

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Tezpur University

* List of equipment purchased indicating the item wise costs may please be provided (only one equipment).

Annexure A

Manpower Staffing Details (In the financial year wise manner)

Year	NAME OF THE PERSON	NAME OF THE POST	DATE OF JOINING	DATE OF LEAVING	TOTAL MONTHLY SALARY	TOTAL SALARY PAID DURING THE FINANCIAL YEAR	TOTAL SALARY PAID DURING PROJECT PERIOD
2019-20	Monalisha Chutia	JRF	16/12/19	18/2/21	31,000/-	1,09,000/-	4,38,928/-
2020-21	Monalisha Chutia	JRF	16/12/19	18/2/21	31,000/-	2,79,000/-	4,38,928/-
	Joydeep Singha	Project Assistant	18/3/21	17/6/21	20,000/-	Nil	2,60,365/-
2021-22	Monalisha Chutia	JRF	16/12/19	18/2/21	31,000/-	50,928/-	4,38,928/-
	Joydeep Singha	Project Assistant	18/3/21	17/6/21	20,000/-	60,365/-(with due draw of 9,032/-)	2,60,365/-
	Joydeep Singha	Project Assistant	1/11/21	31/8/22	20,000/-	1,00,000/-	2,60,365/-
2022-23	Joydeep Singha	Project Assistant	1/11/21	31/8/22	20,000/-	1,00,000/-	2,60,365/-

(U.P. Sarkar) 24/5/23

(Signature of Principal Investigator)

Assistant Professor,
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Tezpur University
Napaam, Tezpur- 784028

(SIGNATURE OF HEAD OF THE INSTITUTE)

Registrar
Tezpur University

(Signature of Accounts Officer)

Finance Officer
Tezpur University

Manpower Expenditure Details (In financial year wise manner)*:

YEAR	SANCTIONED POSTS	NUMBER	SCALE OF PAY	ANNUAL OUTLAY	OUTLAY FOR THE ENTIRE PERIOD	REVISED SCALE, IF ANY	REVISED ANNUAL OUTLAY	REVISED PROJECT OUTLAY	ACTUAL RELASES BY DBT	ACTUAL EXPENDITURE	BALANCE
2019-20	JRF	1	31,000/-	401796/-	1257192	NA	NA	NA	401796/-	109000/-	292796/-
2020-21	JRF	1	31,000/-	401796/-	1257192	NA	NA	NA	Nil	279000/-	13796/-
2021-22	SRF	1	35,000/-	453600/-	1257192	NA	NA	NA	401796/-	211293/-	190503/-
2022-23	SRF	1	35,000/-	453600/-	1257192	NA	NA	NA	Nil	100000/-	90503/-

09/5/23

(Signature of Principal Investigator)

(J. P. Saikia)
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(Signature of Accounts Officer)

Finance Officer
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(SIGNATURE OF HEAD OF THE INSTITUTE)

Registrar
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* Details of manpower salary/ fellowship revision alongwith due- drawn statement and arrears requested should be given separately, if applicable.

Due- Drawn Statement

Name of the Project Staff	Month and Year	Due	Drawn	Difference
Nil				

09/5/23

(Signature of Principal Investigator)

(T. P. Saikia)
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(SIGNATURE OF HEAD OF THE INSTITUTE)

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(Signature of Accounts Officer)

Finance Officer
Tezpur University

UGC Start-Up grant

UTILIZATION CERTIFICATE

It is certified that the amount of Rs.8,00,000/- (Rupees eight lakhs only) released grant out of the total grant of Rs.10,00,000/- (Rupees ten lakhs only) sanctioned to Dr. Jyoti Prasad Saikia by the University Grants Commission vide their letter No. F.30-405/2017 (BSR) dated 09 January 2018 towards UGC-BSR-Start-up grant for newly recruited faculty at Assistant Professor level in science department has been utilized and amount of Rs. Nil/- (Rupees nil only) left as unspent for the purpose for which it was sanctioned and in accordance with the terms and conditions as laid down by the Commission.

If as a result of check or audit objection, any irregularity is noticed at a later stage, action will be taken to refund or regularize the objected amount. We would also like to request release of remaining 20% of sanctioned grant i.e. Rs.(10,00,000-8,00,000)= Rs. 2,00,000/- (Rupees two lakhs only).

Registrar
Tezpur University

Registrar
Tezpur University
(seal)

Finance Officer
Tezpur University

Finance Officer
Tezpur University
(seal)