PROFORMA – I

PROFORMA FOR SUBMISSION OF PROJECT REPORT TO UGC ON COMPLETION OF PROGRAMME SUPPORT

1. Name of the University/ Department : Department of Chemistry:
HNB Garhwal University (A Central University), Uttarakhand
2. State: Uttarakhand
3. Status of the Institute: Centrally funded

Project Title: Natural Products Chemistry

Phytomolecules from selected Himalayan medicinal plants: isolation, characterization and biological activity"

4. Specific Area: **Natural Products Chemistry**

5. Duration: **Five Years**

6. Total Cost (Rs.) **Rs. 58.75Lac**
7. Name of Project Coordinator: Prof. D.S. Negi,
Affiliation: Department of Chemistry, HNB Garhwal University (A central University) , Srinagar (Garhwal) Uttarakhand

8. Project Summary

Concept of the selected project is to identify the herbal active agents from the plant of the Himalayan region through bioassay targeted isolation. Plants occurring at high altitude possess a great extent of medicinal properties; these plants are used as folk medicine to take care of various ailment and diseases. The herbal products represent safety in contrast to the synthetic's product to human as well as environment. India is one of the world's 12 biodiversity centers with the presence of over 45,000 different plant species. In India, drugs of herbal origin have been used in conventional systems of medicines such as *Unani* and *Ayurveda* since ancient times. Traditional systems of medicine carry on being usually skillful on many accounts. Population increases, insufficient supply of drugs, excessive rate of treatments, side effects of several allopathic drugs and development of confrontation to currently used drugs for infectious diseases have led to improved accent on the use of plant resources as a source of medicines for an extensive range of human ailments.. Natural therapies, such as the use of plant natural products in treatment, may reduce adverse side effects. A myriad of many plant products exist that have shown very promising *in vitro*, but have yet to be evaluated in humans. Further study is required to determine the efficacy of these plant products for their use in herbal medicines. . Therefore, it is proposed to contribute to the bioassay targeted isolation of secondary metabolites from the unexplored

medicinal plants for anticancer activity from Garhwal region, which is a vast area of the Himalayan region, blessed with a huge number of medicinal plants.

The Indian Himalayan Region is the section of the Himalayas northern state of Jammu and Kashmir to Himachal Pradesh, Uttarakhand, Sikkim, Arunachal Pradesh, as well as the hill regions of two eastern states, Assam and West Bengal. The central Himalaya region covers the state of Uttarakhand which is divided into major divisions of Kumaon and Garhwal. This region of Himalaya owing to complex topography and verity of climate is sacred with affluent and varied heritage and supports a wide variety of wild and cultivated medicinal and aromatic plants. Folk medicinal plants have attracted a large number of workers, institutions and organizations for their studies and research work in field of medicinal and pharmaceutical sciences to prepare the herbal drugs for the treatment of various curable and non curable diseases as malaria, AIDS, cancer, tuberculosis, diabetes, hypertension, asthma, arthritis, osteoporosis etc.

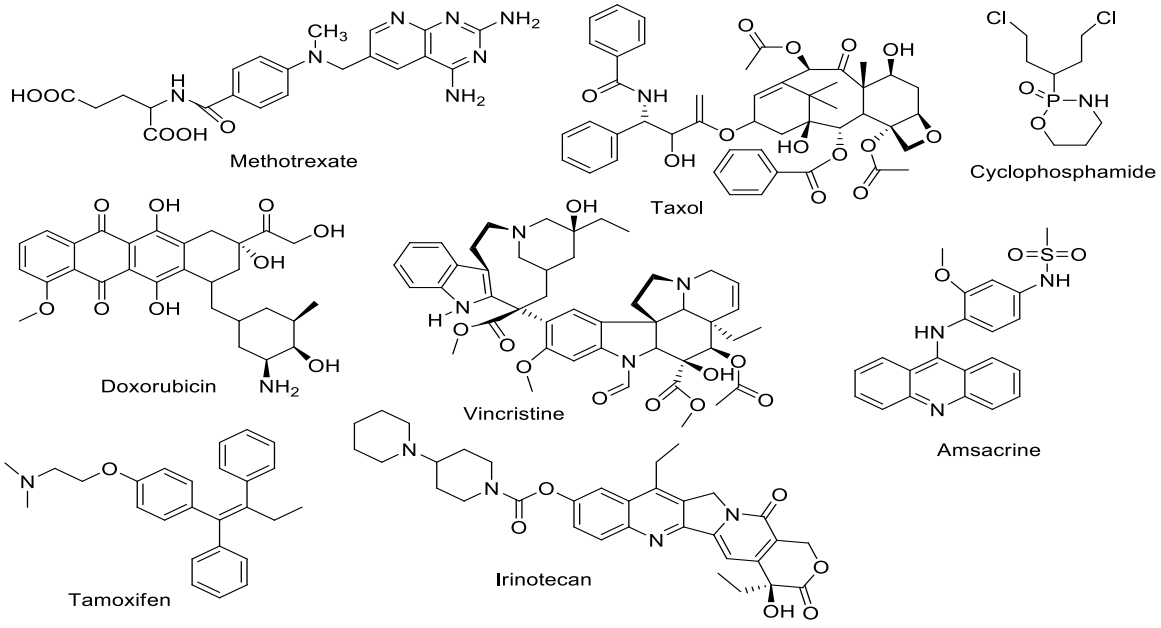


Figure 1: Structure of drugs commonly used in medicine.

These are some most popular drugs used in medicine. Many of them are synthetic drugs or analogues with serious side effects. It is measured that natural products have minimal side effects, little expenses and secure. In this proposal our efforts are also to contribute in search of new/novel or known herbal remedial agents from plant source or to develop herbal formulations that are active against CO-1 and COX 2. Therefore, this study is, intended to investigate biological activity from selected plants.

Aims and Objectives of Study

- ✓ Study of the selected plants of Himalayan region for identification of active principles
- ✓ Collection of plant materials in bulk.
- ✓ Preparation of the plant extract with 90-95% ethanol or other solvents.
- ✓ Bio-assay guided fractionation of the ethanolic extract with hexane, chloroform, n-butanol and water.
- ✓ Screening of extract and fractions for biological activity and with special reference to COX studies
- ✓ Isolation of pure compounds in large quantities from corresponding active fractions using advanced chromatographic techniques HPTLC, Flash Chromatography.
- ✓ Characterization of isolated pure compounds and their activity testing.
- ✓ Possible chemical modification and derivatization of active constituents with their SAR studies.

Table 1: Information of collected plants

S.no.	**Plants**	**Part**	**Collection place**
1.	*Betula utilis*	Bark	Purchased from farmers near Joshimath 3500 m
2.	*Stepania glabra*	Tuber	Rudraprayag- Narkota - 1500 m
3.	*Nyctanthes arbor-tristis*	Leaves	Srinagar- Garhwal - 500 m
4.	*Lantana camara*	Leaves	Srinagar- Garhwal 500m

During last 10 years the PI has carried out the bioactive guided isolation of secondary metabolites from medicinal plants of Uttarakhand. Himalayan region and published results in peer reviewed journals. He has worked on four different projects as PI funded from DST, UGC, CSIR and UCOST in the area of medicinal plants having different biological activity, also underwent training in the area of synthetic chemistry at School of Manchester, UK (02 years as Post Doc). PI has established links with Institute like CSIR- IHBT, Palampur, Era's Medical College, Lucknow; Bose Institute, Kolkata; CDRI, Lucknow; NCL, Pune where the biological activity and synthesis of analogues of active compounds can be undertaken.

Preparation of the crude extract of the plant material

The freshly collected plant material is dried in shade in a well ventilated enclosure. Dried plant material should be powdered and placed in a percolator of appropriate size. The plant material is then submerged in 95% alcohol (Alcohol has been found to be a good, cheep and all purpose solvent for preliminary extraction). After standing for about 16 hours (overnight) at room

temperature, the alcoholic extract is drained off. This process of extraction at ambient temperature is repeated five times which is generally sufficient for exhaustive extraction of the plant material. The combined alcoholic extract is evaporated to dryness under reduced pressure below 50^0. In case of plants containing alkaloids, extraction may be carried out with alcohol containing 5% acetic acid.

If the pharmacological evaluation of the fresh plant material is desired, then the freshly collected plant material is immersed in alcohol without much loss of time so as to prevent any enzymatic transformation of the constituents during storage. Its extract is prepared in the same manner as described above. After preliminary bioassay guided fractionation of the alcoholic extract and ascertaining the polarity, chemical nature and thermolability of the bioactive constituents, the subsequent extraction procedure may be modified according to the need. For highly non-polar compounds n-hexane may be used as the solvent of extraction while for highly polar compounds 50% aqueous alcohol or water is the solvent of choice. In case of thermally labile compounds evaporation of the solvent is carried out under reduced pressure at ambient temperature or preferably by freeze drying (aqueous extracts). Before evaporation, the aqueous extract should be stored in a cold room with addition of appropriate quantity of toluene so as to prevent any fungal growth. The dried aqueous extract should be stored in a deep freeze.

Fractionation

The alcoholic extract of the plant is a mixture of a large number of chemical constituents of wide ranging polarities. These constituents may be separated into several groups of compounds of similar polarities by fractionation with solvents of different polarities. Thus, dried alcoholic extract is first macerated four times with n-hexane. The residue is then macerated four times with chloroform. The remaining insoluble portion is suspended in water and extracted four times with n-butanol. Any insoluble matter suspended in the aqueous layer is filtered off. The n-hexane, chloroform, n-butanol and water soluble fractions are evaporated to dryness under reduced pressure below 50^0. The fractionations obtained above are subjected to pharmacological evaluation. The active fraction is then taken up for gross separation by appropriate methods, e. g.

i. Acid base separation
ii. Gross chromatography (collecting only 5 to 6 fractions) over silica gel, alumina, XAD-resin, ion-exchange resin or polyamide, etc.

Sub-fractions so obtained may be further evaluated for their biological activity and the active fraction(s) may be taken up for the isolation of active principle(s) by further bioassay guided fractionation using finer separation techniques such as preparative HPLC, **Flash**

chromatography (over normal, reverse phase or chiral columns), gel permeation, ion-exchange, polyamide chromatography.

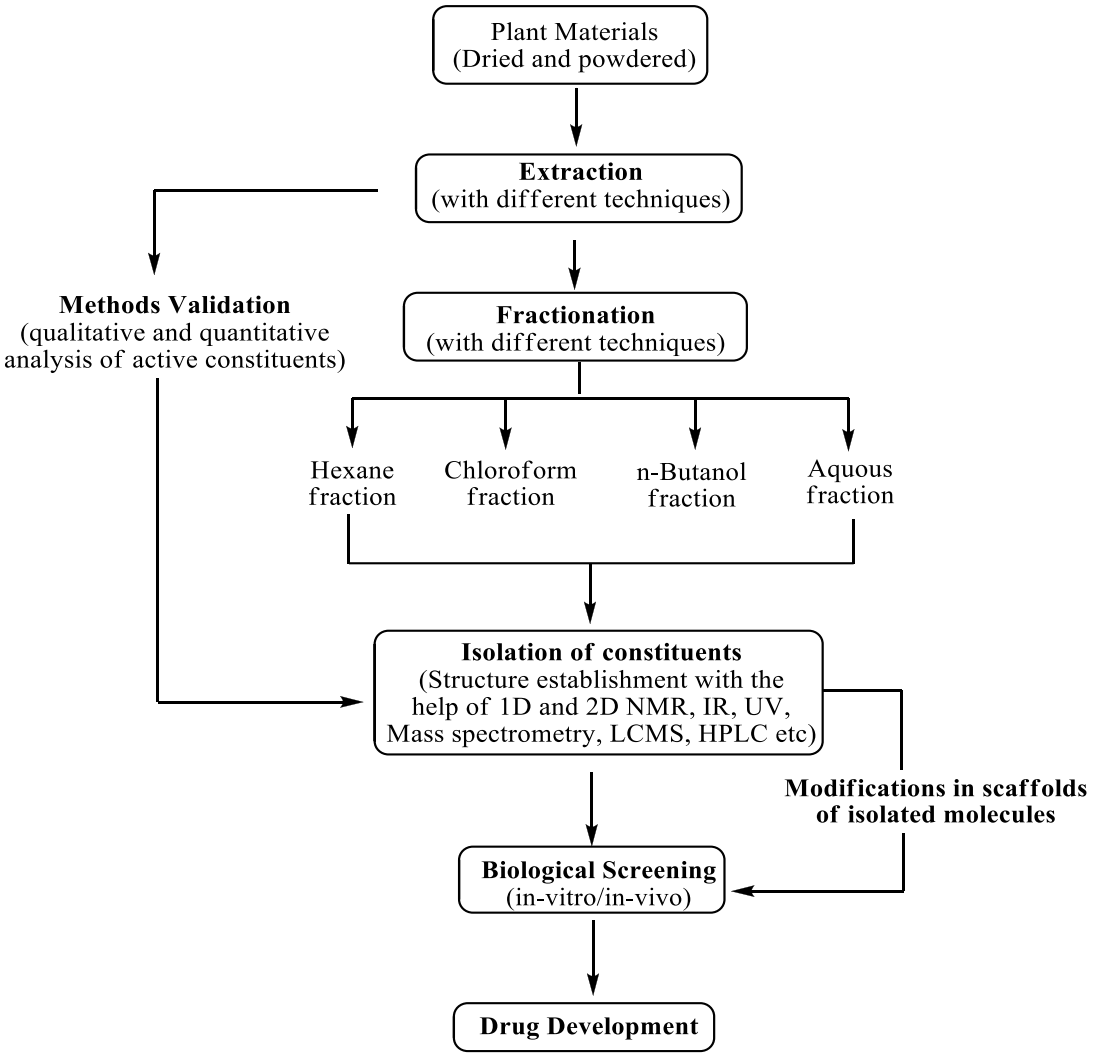
Bibliography:

i. Screening of Indian Medicinal Plants for Biological Activity, Pt. I. M. L. Dhar, M. M. Dhar, B. N. Dhawan, B. N. Mehrotra and C. Ray, *Ind. J. Exp. Biol*. 6, 231 (1968).

ii. J. B. Harborne (1973), *Phytochemical Methods*, pp1, Chapman and Hall, London.

2 Methodology and techniques

Flow chart 1: Schematic representation of extraction and fractionation of collected plant material.



Following techniques will be used in isolation and characterization and biological screening

- ✓ **Thin Layer Chromatography:**

Thin Layer Chromatography (TLC) is used for the detection of constituents, for monitoring the column chromatography and for testing the purity of the isolates. Spraying reagents (5%alcoholic

H_2SO_4 solution, H_2SO_4-vanillin etc.,) and UV visible light are used to detect the components present on pre-coated silica gel TLC of Merck company.

✓ **Chemical Tests:**

Neutral $FeC1_3$ for Phenolic compounds, Shinoda test for flavonoids, Dragendorff reagent for alkaloids and Lieberman Buchard test for terpenoids etc., to be carried out to know the nature of the compounds.

✓ **Column Chromatography:**

Column Chromatography has to be done by standard procedures. Silica Gel-H and silica-gel for column chromatography 60-120, 100-200 and 230-400 mesh are used as an adsorbent. The material to be chromatographed, adsorbed on an adequate quantity of silica gel is dried and loaded on to the column. Different column techniques like flash column, Dry Column (DC), Vacuum Liquid Chromatography (VLC) and HPLC are used to separate the components.

✓ **Characterization:**

Structure elucidation of the bioactive compounds could be done by various spectral data

IR: Infrared spectrum is useful to know the information of functional groups.

UV: Ultraviolet spectrum is useful to know the unsaturation and nature of compound.

PMR: Proton magnetic resonance spectrum (^1H) is useful to know the number of protons and nature of protons.

CMR: Carbon magnetic resonance spectrum (^{13}C and DEPT) gives information regarding the nature of carbon and its number.

2D-NMR: Two-dimensional nuclear magnetic resonance spectrum (COSY, NOESY, HSQC, HMBC etc.,) gives information regarding the attachment of protons to their concerned carbons.

Mass: Mass spectral data gives the possible fragmentation pattern and its molecular weight.

Research labs of Department of chemistry are well equipped, having facilities for research purpose.

We also have computer labs with 24 hrs. Internet facilities for quick literature search.

1. Equipments purchased: The major grant of Rs 40.0 Lac was to purchase the equipment. Two years from 2020 to 2022 work could not be done because of the pandemic. UGC has been requested for further extension of the project. The equipment was purchased through GeM portal following the GFR 2017 rules. The PhD students and M Sc students have utilized the equipments for their PhD work and the department has also trained 35 M Sc and PhD students from other departments of the university, colleges and other state and central universities and

institutes. 01 week training programme was organized for students sponsored by DST- STUTI in May 2022.

S. No.	Name of Equipment purchased	Year of Purchase	Make
1	Flash Chromatography (Buchi)	2022 thorugh GeM – GFR-2017	Buchi,Switzerland

Internal monitoring and evaluation mechanism:

University has separate cell of Audit from CAG, Govt. of India, Accounts Division headed by Finance Officer and Deputy Registrar (Finance) monitors the grants released by funding agencies. Beside Departmental Committee comprising of Departmental members and Finance Officer, Registrar and CA also are involved. Gem and GFR is strictly followed to utilize the grants.

Outcome of the project

There are many plants for which intrinsic anti-inflammatory activity is an anecdote from other known pharmacological activities related to the variation of the complex inflammatory response. At present, there is escalating scientific evidence for the anti-inflammatory activity of many plants. There are many plants for which the anti-inflammatory activity has been widely studied while primary sign has been recognized for others. There are many phytochemicals which had been isolated structurally and pharmacologically characterized as an anti-inflammatory remedy. But many plants show anti-inflammatory activity while their COX inhibition property is not well recognized. So, further analyses for COX inhibition for some selected plants were done in the thesis work.

Based on the literature survey plants (*Stephania glabra, Betula utilis, Nyctanthes arbor-tristis* and *Lantana camara)* were selected and collected. Plants were identified with the help of experts. The collected plant was dried and powdered and powdered. Dried and powdered plant materials (Tuber of *Stephania glabra,* leaves of *Lantana camara, Nyctanthes arbor-tristis* and bark of *Betula utilis*) were extracted with four different solvents by using a Soxhlet extractor and four different extracts i.e. hexane, chloroform, ethyl acetate and methanolic extract obtained for each plant material, which is dried and screened for their in COX-1/2 inhibitory and DPPH radical scavenging activity. The in-vitro analysis of all four extracts of the tuber of *Stephania glabra* shows that chloroform extract has greater potential to inhibit COX-2 as compared to celecoxib and other extracts. The in-vitro analysis of all four extracts of the leaf of *Lantana camara* shows that chloroform extract has greater potential to inhibit COX-2 as compared to celecoxib and other extracts. The in-vitro

analysis of all four extracts of bark of *Betula utilis* shows that chloroform fraction has greater potential to inhibit the COX-2 as compared to celecoxib and other extracts. The in-vitro analysis of all four extracts of the leave of *Nyctanthes arbor-tristis* shows that the methanolic fraction has the greatest potential to inhibit the COX-2 as compared to other extracts.

The result of the COX-1&2 inhibition assay shows that chloroform extract from *Stephania glabra* and methanolic extract from *Lantana camara*, *Betula utilis*&*Nyctanthesarbortritis* possess the highest inhibitory activity against COX-2. However, the chloroform extract from *Stephania glabra* shows the best inhibition property against COX isoenzyme.

The molecular docking study has been done for the reported phytochemicals of all four plants. The designing of all compounds was done by using ACD Chemsketch and Chem Draw 12.0 ultra. The groundwork of protein was done using UCSF Chimera 1.11.2 & Discovery studio visualizer v 3.5. in the dock prep module. The docking of all reference compounds and the selected phytochemicals of all four plants was performed using PyRx&iGEM DOCK software against COX-1 (PDB ID – 3KK6) and COX-2 (PDB ID – 3LN1). Vina Wizard docking script was applied for the docking process in PyRx. Open Babel for importing SDF files, removing salts and energy minimization. The post-dock modeling of docked poses was executed by Discovery studio visualizer v 3.5.In order to know the drug likeliness of molecule/ligand PKCSM and Swiss ADME online platform were used. The MD simulations were conceded out by means of Desmond simulation package of Schrodinger LLC.

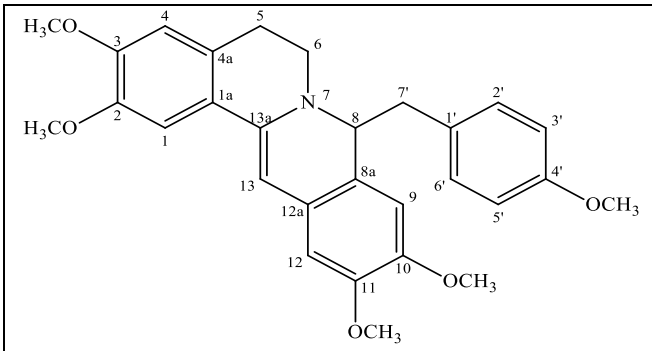
The molecular docking study of reported phytochemicals from the tuber of*Stephania glabra* shows that compounds 8, 9, 10, 13, 14, 16 and 24 possess a great inhibition potential based on docking score against COX-1 and COX-2 therefore, an in-vitro comparative study is required for compound 8, 9, 10, 13, 14, 16 and 24 against COX-1 and COX-2 to conclude the selective and potential COX-2 inhibitor for the development of new anti-inflammatory drug without causing any gastrointestinal ulcer to provide a good therapeutic option to treat the inflammation.

The molecular docking study of reported phytochemicals from the leaf of *Lantana camara* also shows that compounds 1', 6', 7', 8', 30' and 32' possess a great docking score against COX-1 and COX-2 while ligands 30' and 1' shows selectivity for COX-2 over COX-1 therefore, an in-vivo comparative study is required for these compounds against COX-1 and COX-2 to conclude the selective and potential COX-2 inhibitor for the development of the new anti-inflammatory drug.

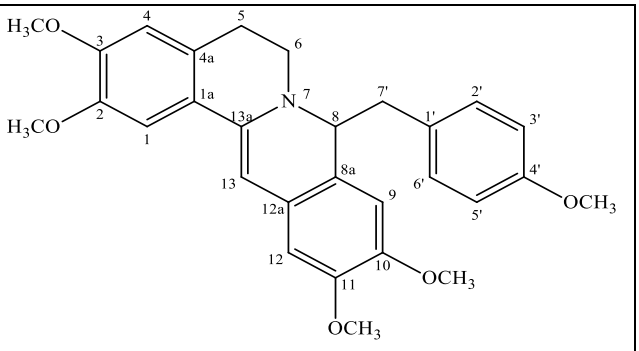
The molecular docking study of reported phytochemicals from the bark of the *Betula utilis* also shows that compounds 16'', 17'', 18'', 19'' and 21'' possess a great inhibition potential against COX-1 and COX-2 but ligands 21'' and 22'' shows docking selectivity for COX-2 express the novelty for finding good NSAIDs. The molecular docking study of reported phytochemicals from the leave of the *Nyctanthes arbor-tristis* also shows that compounds 6*, 21*, 2* and 1* have the highest binding affinity/docking score for 3KK6 (COX-1 enzyme) while ligands 14*, 1(-10.2 kcal/mol), 2* and 6* have highest binding affinity for 3LN1 (COX-2 enzyme) but compound 1*, 2* and 14* also shows docking selectivity for COX-2 express the novelty for finding good NSAIDs.F urther the MD simulation study reveals the stability of protein-ligand complex, conformational change in protein and ligand during docking process. The MD simulation also predict the rGyr (radius of gyration of ligand molecule), IHB (intramolecular hydrogen bond), MolSA (molecular surface area), SASA (solvent-accessible surface area) and PSA (polar surface area).

In additional work,the antioxidant activity of all extracts from the selected plant was carried out by DPPH radical scavenging method were done. The result of anti-oxidant activity shows that the Ethyl acetate extract of *Lantana camara*, *Betula utilis*, *Nyctanthesarbortritis*& MeOH extract of *Stephania glabra* possess the highest DPPH radical scavenging property.

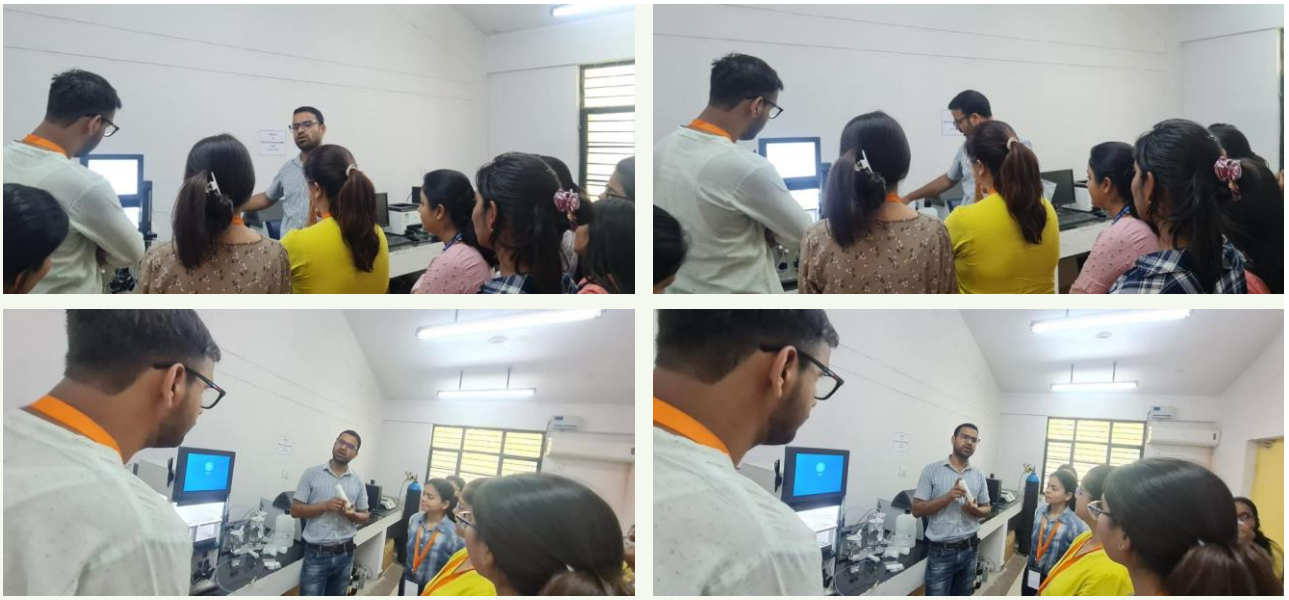
Attempts were made to isolate the phytochemicals from *Stephania glabra* and Nyctanthes *arbortritis.* The compound SG1 & SG2 are isolated from the tuber of the plant *Stephania glabra*.The instruments and techniques used for the characterization of phytochemicals are Double beam UV-Visible Spectrometer (Elico SL 218), HPTLC -CAMAG, Flash Chromatography – Buchi , Bruker Advance-400 (400 MHz 195 for 1H-NMR and 100 MHz for 13C-NMR), Agilent Cary 630 FTIR Spectrometer (Range: 4000- 196 450 cm-1) and Agilent 6520 Q-TOF Mass Spectrometer.



Structure of Compound SG1: 2,3,10,11-tetramethoxy-8-(4'-methoxybenzyl)-5,6-dihydro-6H-isoquinolino[3,2-α]isoquinoline or 8-(4' -methoxybenzyl) xylopinine

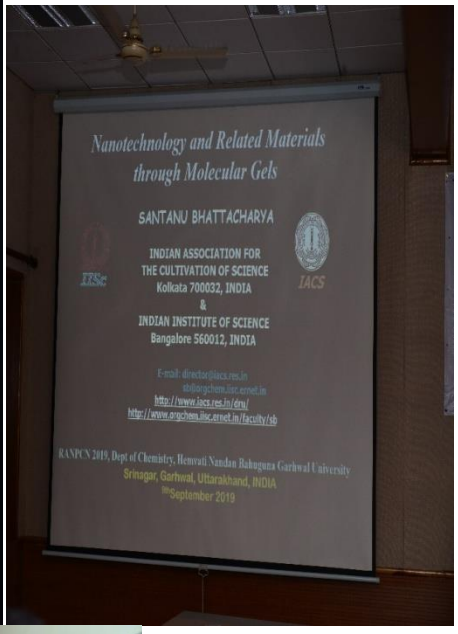


Structure of Compound SG2: 2,3,10,11-tetramethoxy-8-(4'-hydroxybenzyl)-5,6-dihydro-6H-isoquinolino[3,2-α]isoquinoline or 8-(4' -hydroxybenzyl) xylopinine



(Pics of training in the department on the instruments during one week DST- STUTI programme)

(Pics of National Conference organized by Department of Chemistry during September 9-10 , 2019 on the theme of the UGC –SAP project (Natural Product Chemistry) the seminar was sponsored by UGC and other funding agencies) The seminar was inaugurated by S.S. Bhatnagar prize awardee Prof. Santanu Bhattacharya FNASc FASc FNA FTWAS , Department of Organic Chemistry, Indian Institute of Science Bangalore- Director IACS Kolkata and Presently Director IISER- Tirupati and Hon'ble Vice Chancellor of the university Prof. Annpurna Nautiyal)



FINAL REPORT IS YET TO BE SUBMITTED. REQUEST IS MADE TO UGC TO EXTEND THE PROJECT BECAUSE OF THE COVID PANDEMIC- 2019