CSIR-IIIM

वार्षिक प्रतिवेदन
Annual Report
2015-2016

सीएसआईआर–भारतीय समवेत औषध संस्थान, जम्मू–180001 (भारत)
CSIR-Indian Institute of Integrative Medicine
(Council of Scientific and Industrial Research)
JAMMU-180001 (INDIA)

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DIRECTORS REPORT

It is my proud privilege to present the Annual Report of CSIR-Indian Institute of Integrative Medicine, Jammu to its readers which highlights the major scientific achievements and work done at IIIM during the year 2015-2016. Every year this institute publishes its Annual Report based on the research activities in its core area of research and development. The strength of CSIR-IIIM has always been in medicinal chemistry, drug discovery, natural products chemistry that includes plants and microbes and plant bioresource sciences. I am indeed happy to inform that the strides of progress have continued unabated towards excellence in research and development of innovative products for societal benefit. This period has been highly exciting for us as IIIM, Jammu filed 21 patents applications both in India and in foreign countries and seven patent were granted to IIIM. During this period, IIIM published a total of 141 scientific publications with an average impact factor of 3.528.

During this period extensive extension activities for cultivation and processing of Medicinal and Aromatic plants based on CSIR technologies have been taken up to farmers field to provide alternative and additional source of income and employment opportunities in rural farming sector under JAAG Project. Four CSIR laboratories such as CIMAP, IHBT, NEIST and IIIM are pooling their resources and agro-technologies in the field to motivate the farmers for the cultivation of aromatic grasses. Under this project extensive field survey for identification of rainfed and waste land, signing agreements with various farmers, cultivating trainings, industry linkage, value addition has been taken up in districts of Kathua,Udhampur, Bhadarwah, Doda, Kishtwar and Reasi in Jammu region of J&K state . It is expected that for rural and backward farmers, the cultivation of aromatic grasses will change the economy of the poor farmers and the state as a whole.

During this period two scientist of our institute received national honours and award which include *NASI Platinum Jubilee Young Scientist Award* in Chemical Sciences and the *CSIR Young Scientist Award* in Chemical Sciences for the significant contributions in the field of natural products and development of new synthetic methodologies for bio-active molecules.

I am happy to inform that IIIM scientists developed yet another health drink based on Seabuckthorn a berry, abundantly available in Leh-Ladadh region. An entrepreneur from Leh has taken the entire lot produced by IIIM for market survey and feedback so as to commercially produce the health drink from seabuck thorn in large scale during next fruiting season.

I extend my gratitude to all the staff members both from the scientific and administrative streams of our institute for their year-long dedicated and sincere work and for their cooperation in maintaining the R&D growth of IIIM, Jammu. I believe that the untiring dedication for work offered by my colleagues will take this institute to new heights in coming days.

(Ram Vishwakarma)

1.0 BIODIVERSITY AND APPLIED BOTANY

Formulation and implementation of J&K Aroma Arogya Gram project in the state of Jammu & Kashmir to focusing on better economic and employment generation for the rural masses of J&K state.

Covered about >50.0 hectare of cultivated land with cultivation of CSIR agrotechnologies (*Cymbopogon* sps., Mint sps., Lavender, Rose, Rosemary, *Monarda citriodora*) for better economical return over traditional crops. Ten districts Kathua, Udhampur, Reasi, Ramban, Poonch, Doda, Rajouri, Kishtwar Jammu, Samba of J&K is covered under the project. More than 150 nos. of farmers beneficiaries are registered under the project .

2. PLANT BIOTECHNOLOGY AND DIVERSITY

2.1. Field surveys and tours for collection and documentation of plants

Bikarma Singh

Plant collection, plantation & documentation field tours were conducted in the interior regions of Jammu & Kashmir State for various R & D activities undertaken at CSIR Indian Institute of Integrative Medicine. A brief summary for this type of task undertaken during April 1, 2015 to March 31st, 2016 are given below:

Area surveyed (district)	Objective(s)	Outcome(s)	Period
Patnitop (Udhampur district)	Survey and collection of target plant, *Bergeniaciliata*(Haw.) Sternb. (Saxifragaceae), for DNA barcoding, tissue culture and experiment for captive cultivation	Samples DNA bar-coded and tissue culture initiated by sister division, 87 samples of live plants experiments in progress for cultivation	April 2015
Uttarbehni (Samba district)	Survey and collection of plant samples during forest mapping and biodiversity study	Collected 72 samples were taxonomically identified and voucher deposited in RRLH	April 2015
Reasiproper and surrounding areas (Reasi district)	Collection of target plant, *Vitexnegundo* L. (Verbenaceae), for biochemical screening	Samples DNA bar-coded by sister division	April 2015
Nandini Wildlife Sanctuary(Udhampur district)	Collection of target plant, *Mallotusphilippensis*(Lam.) Mull.-Arg. (Euphorbiaceae), for chemical profiling and biochemical screening	Chemical profiling and biochemical screening	July 2015
Gurez valley (Bandipora district)	• Floristic composition and Forest mapping • Ethnobotanical documentation on medicinal & aromatic plants • Wild edible plants used by *Sheenas*	Plant diversity study and distribution, herbarium enrichment, folk knowledge information	August 2015
Basoli (Kathuwa district)	Survey and plantation of targeted aromatic and medicinal crop Java citronella	Survey undertaken and plantation completed	September 2015
Pallan area	Survey and plantation of	Survey undertaken	September

(Kathuwa district)	targeted aromatic and medicinal crop Java citronella	and plantation completed	2015
Area surveyed (district)	**Objective(s)**	**Outcome(s)**	**Period**
Batote and surrounding areas (Udhampur district)	Collection of different medicinal psychoactive plants, for Chemical Screening	Chemical evaluation is under progress by sister division	October 2015
Purmandal areas (Samba district)	Collection of target plant, *Butea monosperma* (Lam.) Taub. (Fabaceae), for biochemical screening	Biochemical evaluation	January 2016
Pallan and surrounding areas (Samba district)	Survey and plantation of targeted aromatic and medicinal crop Java citronella	Survey undertaken and plantation completed	February 2016
Purmandal and surrounding areas (Samba district)	Survey and plantation of targeted aromatic and medicinal crop Java citronella	Survey undertaken	March 2016
Drub area (Jammu district)	Survey and plantation of targeted aromatic and medicinal crop Java citronella	Survey undertaken	March 2016
Mahanpura (Kathuwa district)	Survey and plantation of targeted aromatic and medicinal crop Java citronella	Survey undertaken and plantation completed	March 2016
Zeeri and surrounding areas (Reasi district)	Survey and plantation of targeted aromatic and medicinal crop Java citronella	Survey undertaken and plantation completed	March 2016

2.2. Plant collection for chemical screening of psychoactive compounds

Bikarma Singh

The psychoactive drugs are chemical substances that can changes brain function and results in alterations in perception, mood, or consciousness. Psychoactive substances when taken in or administered into one's system, directly affect mental processes. During 2015-2016, forty six plant parts of 37 different species from different locations were collected, authenticated and voucher samples maintained at RRLH for future reference. The details of plant samples supplied in powdered form are given below.

Botanical name	Family	Part(s) collected	Quantity (dry powder)
***Acacia modesta* Wall.**	Mimosaceae	Bark	9.9 gm
***Acacia nilotica* (L.) Delile**	Mimosaceae	Bark	17.1 gm
***Acacia nilotica* (L.) Delile**	Mimosaceae	Leaves	20 gm
***Achilleamillefolium*L.**	Asteraceae	Whole plant	11 gm
***Achyranthesbidentata* Blue**	Amaranthaceae	Aerial parts	15 gm
***Aconitum violaceum* Jacq. exStapf.**	Ranunculaceae	Aerial parts	9.5 gm

***Ageratum conyzoides* (L.) L.**	Asteraceae	Whole plant	12 gm
***Berberis lyceum*Royle**	Berberidaceae	Aerial parts	8.4 gm
***Bombaxceiba*L.**	Bombaceae	Leaves	14 gm
***Calotropisprocera*(Aiton) Dryand.**	Apocynaceae	Aerial parts	9 gm
***Cannabis sativa* L. (Jammu)**	Cannabaceae	Aerial parts	7.5 gm (Male)
***Cannabis sativa* L. (Jammu)**	Cannabaceae	Aerial parts	8.2 gm (Female)
***Cannabis sativa* L. (Kathua)**	Cannabaceae	Aerial parts	5.0 gm (Mixed)
***Cannabis sativa* L. (Nandini WLS)**	Cannabaceae	Aerial parts	8.7 gm (Mixed)
***Cannabis sativa* L. (Vijayapura)**	Cannabaceae	Aerial parts	2.9 gm (Male)
***Cascabellathevetia*(L.) Lipp.**	Apocynaceae	Leaves	17.6 gm
***Catharanthus roseus* (L.) G.Don**	Apocynaceae	Aerial parts	8 gm
***Cryptolepisbuchananii*Roem&Schult**	Apocynaceae	Aerial part	12 gm
***Datura innoxia*Mill.**	Solanaceae	Fruits	20 gm
***Euphorbia helioscopia*L.**	Euphorbiaceae	Aerial parts	11gm
***Euphorbia hirta* L.**	Euphorbiaceae	whole plant	16.5 gm
***Ipomoea cairica* Sweet**	Convolvulaceae	Aerial parts	8.3 gm
***Jacaranda mimosifolia*D.Don**	Mimosaceae	Leaves	23.2 gm
***Justiciaadhatoda* L.**	Acanthaceae	Stem	20 gm
***Kigeliaafricana* (Lam.) Benth.**	Bignoniaceae	Fruits	23.9 gm
***Kigeliaafricana*(Lam.) Benth.**	Bignoniaceae	Bark	8.2 gm
***Lantana camara*L.**	Verbenaceae	Leaves	12.5 gm
***Magnolia hodgsonii*Hook.f. & Thom.**	Magnoliaceae	Leaves	10 gm
***Melia azedarach* L.**	Meliaceae	Fruits	40 gm
***Melia azedarach* L.**	Meliaceae	Leaves	8 gm
***Mirabilis jalapa*L.**	Nyctaginaceae	Aerial parts	18 gm
***Mitragynaparviflora*(Roxb.) Korth.**	Rubiaceae	Stem bark	5.9 gm
***Nerium oleander* L.**	Apocynaceae	Leaves	17 gm
***Nicotiana plumbaginifolia* Viv.**	Solanaceae	Whole plant	15 gm
***Partheniumhysterophorus*L.**	Asteraceae	Aerial parts	20 gm
***Phyllanthusamarus*Schum. &Thonn.**	Euphorbiaceae	Whole plant	3.5 gm
***Ricinus communis* L.**	Euphorbiaceae	Leaves	30 grams
***Senna occidentalis*(L.) Link.**	Caesalpinaceae	Whole plant	10 gm
***Solanum torvum*Sw.**	Solanaceae	Leaves	20 gm
***Tabernaemontana divaricata* R.Br**	Apocynaceae	Bark & stem	12 gm
***Tabernaemontana divaricata* R.Br**	Apocynaceae	Leaves	20 gm
***Tabernaemontana divaricata* R.Br**	Apocynaceae	Flowers	12 gm
***Tecomastans* (L.) Kunth.**	Bignoniaceae	Leaves	43.2 gm
***Urticadioica*L.**	Urticaceae	Leaves	15gm
***Withaniasomnifera*(L.) Dunal**	Solanaceae	Aerial parts	10 gm

2.3 Collection of plant materials in bulk quantity for chemistry

Bikarma Singh

During 2015-2016, bulk quantity of 8 plant species were collected from different regions of Himalaya from different growing seasons. The plants were authenticated and voucher sample maintained for future reference. The details of plant collected during the reporting period are given below:

Botanical name / Family	Parts supplied / CDR Code	Quantity
***Aconitum violaceum*Jacquem. exStapf. / Ranunculaceae**	Whole part / P13	250 gm dried weight
***Callistemon citrinus*(Curtis) Skeels / Myrtaceae**	Bark / P10	4.0 kg dried weight
***Cannabis sativa* L. / Cannabaceae (Mixed)**	Aerial part / P08	3.0 kg dried weight
***Cannabis sativa* L. / Cannabaceae (Male)**	Aerial part / P08	4.5 kg dried weight
***Cannabis sativa* L. / Cannabaceae (Female)**	Aerial part / P08	2.5 kg dried weight
***Croton bonplandianus* Baill. / Euphorbiaceae**	Aerial part / P08	350 gm dried weight
***Glycyrrhizaglabra* L. / Fabaceae**	Stem / P02	350 gm fresh weight
***Mallotusphilippensis* (Lam.) Müll.Arg. / Euphorbiaceae**	Leaves/ P03	1.5 kg dried weight
***Mallotusphilippensis* (Lam.) Müll.Arg. / Euphorbiaceae**	Bark /P10	4.0 kg dried weight
***Neolamarckiacadamba*(Roxb.) Bosser / Rubiaceae**	Bark / P10	3.7 kg dried weight
***Phyllanthusamarus*Schum. &Thonn. / Euphorbiaceae**	Whole part / P13	350 gm dried weight

2.4 Collection of plant materials for DNA barcoding

Bikarma Singh

During 2015-2016, Thirty two different accessions of plant samples were collected from different regions of Kashmir Himalaya for DNA barcoding under different project undergoing at IIIM. These plants were authenticated and a voucher samples were maintained for future reference. The details of the plants collected along with GPS coordinates are given below:

Sr No	Botanical Species	Family	Location	Coordinates		
				Longitude	Latitude	Elevation
1.	*Artemisia maritima* L. (Sample 1)	Asteraceae	Kanzalwan	N34°38.034″	E74°50.330″	2432 m
2.	*Artemisia maritima* L. (Sample 2)	Asteraceae	Gurez	N34°37’46.5″	E74°52’22.7″	2449 m
3.	*Artemisia dracunculus* (Sample 1)	Asteraceae	Kanzalwan	N34°38.034″	E74°50.330″	2432 m
4.	*Artemisia dracunculus* (Sample 1)	Asteraceae	LoC Badgaw	N34°33.528″	E75°02.579″	2694 m
5.	*Prangospabularia*Lindl.	Apiaceae	Gurez	N34°37’46.5″	E74°52’22.7″	2449 m
6.	*Betulautilis* Jacq.	Betulaceae	Razdan Pass	N34°34.239″	E74°38.375″	2972 m
7.	*Valerianajatamansis* Jones(Sample 1)	Valerianaceae	Pahalgam	N34°04.875″	E75°15.956″	2384 m
8.	*Valerianajatamansis* Jones(Sample 2)	Valerianaceae	Razdan Pass	N34°34.239″	E74°38.375″	2972 m
9.	*Valerianajatamansis*Jones (Sample 3)	Valerianaceae	Gurez	N34°37’46.5″	E74°52’22.7″	2449 m
10.	*Heraculumcandicans*	Apiaceae	Yarika	-	-	-

	Wall. ex DC. (Sample 1)					
11.	*Heraculumcandicans*Wall. ex DC. (Sample 2)	Apiaceae	Pahalgam	N34°04.875″	E75°15.956″	2384 m
12.	*Heraculumcandicans*Wall. ex DC. (Sample 3)	Apiaceae	Lidder Valley	N34°04.896″	E75°15.971″	2399 m
13.	*Heraculumcandicans*Wall. ex DC. (Sample 4)	Apiaceae	Green house Pahalgam	N34°04.875″	E75°15.956″	2384 m
14.	*Heraculumcandicans*Wall. ex DC. (Sample 5)	Apiaceae	Gurez	N34°37'46.5″	E74°52'22.7″	2449 m
16.	*Berberislycium*Royle(Sample 1)	Berberidaceae	Gurez	N34°37'46.5″	E74°52'22.7″	2449 m
17.	*Angelica archiangelica* L. (Sample 1)	Apiaceae	Pahalgam	N34°04.875″	E75°15.956″	2384 m
18.	*Angelica archiangelica* L. (Sample 2)	Apiaceae	Gurez	N34°37'46.5″	E74°52'22.7″	2449 m
19.	*Angelica archiangelica*L. (Sample 3)	Apiaceae	Kanzalwan	N34°38.034″	E74°50.330″	2432 m
20.	*Angelica archiangelica* L. (Sample 4)	Apiaceae	Razdan Pass	N34°34.239″	E74°38.375″	2972 m
21.	*Angelica archiangelica* L. (Sample 5)	Apiaceae	Srinagar	N34°21.001″	E74°39.541″	1641 m
22.	*Bergeniaciliata*(Haw.) Sternb.	Saxifragaceae	Tulel	-	-	-
23.	*Hyocymusniger* L(sample 1)	Solanaceae	Gurez	N34°37'46.5″	E74°52'22.7″	2449 m
24.	*Hyocymusniger* L(sample 2)	Solanaceae	Gurez	N34°37'46.5″	E74°52'22.7″	2449 m
25.	*Aconitum heterophyllum*Wall. (Sample 1)	Ranunuculaceae	Pahalgam	N34°04.875″	E75°15.956″	2384 m
26.	*Aconitum heterophyllum*Wall.(Sample 2)	Ranunuculaceae	Razdan Pass	N34°34.239″	E74°38.375″	2972 m
27.	*Aconitum heterophyllum*Wall.(Sample 3)	Ranunuculaceae	Kanzalwan	N34°38.034″	E74°50.330″	2432 m
28.	*Aconitum heterophyllum* Wall.(Sample 4)	Ranunuculaceae	4 km down Razdan Pass	N34°36.985″	E74°53.062″	2472 m
29.	*Aconitum heterophyllum* Wall.(Sample 5)	Ranunuculaceae	Srinagar green house	N34°21.001″	E74°39.541″	1641 m
30.	*Hypericumperforatum* L.	Hypericaceae	LoC Badgaw	N34°33.528″	E75°02.579″	2694 m
31.	*Heraculeumcandicans* Wall. ex DC. (Sample 1)	Apiaceae	Yarika	-	-	-
32.	*Heraculeumcandicans* Wall. ex DC. (Sample 2)	Apiaceae	Srinagar	N34°21.001″	E74°39.541″	1641 m

2.5 Bioresourceinventorization with focus on bioprospection of Gurez valley

Bikarma Singh

During 2015-2016, one field tour to Gurez valley was carried out *w.e.f.* 24th-30th August 2015 for inventorization of plant diversity and resource mapping. During surveys, visited 13 different localities, collected 176 voucher samples along with field notes (date of collection, habit, cal ecologinotes, notes on ethnobotany, local name, part used etc.) and GPS points (altitude, longitude and longitude). Total 213 species have been identified so far from the valley and more than 1000 digital photographs of different plants and their parts were taken for species

authentication and writing description. Essential oil yielding plants such as *Artemisia dracunculus* L., *Artemisia vulgaris* L. And *Nepeta cataria* L. were collected from the valley, their oil extracted and compound identification is in progress.

Figure 2.5.1: Inventorization and Mapping of Critically Endangered Medicinal Plant Species in Gurez Valley of Kashmir Himalaya;(A) *Swertiapetiolata*D.Don (Gentianaceae), (B) *Aconitum chasmanthum* Stapf ex Holmes. (Ranunculaceae), (C) *Jurineamacrophala*Benth.(Asteraceae), (D*) Sinopodophyllumhexandrum* (Royle) T.S.Ying (Berberidaceae), (E*) Aconitum heterophyllum* Wall.(Ranunculaceae), (F) *Picrorhizakurroa*Royle ex Benth.(Plantaginaceae).

Figure 2.5.2 Extraction of essential crude oil from wild aromatic plants growing in Gurez valley of Kashmir Himalaya

2.6. Ethnobotany, Traditional Knowledge, and Diversity of Wild Edible Plants and Fungi: A Case Study in the Bandipora District of Kashmir Himalaya, India

Bikarma Singh, P. Sultan, Q.P. Hassan, S. Gairola, Y.S. Bedi

Tribals living in Himalaya are endowed with a strong culture of herbal usage and have an ancestral practice with regard to the use of wild plants as food and medicines. This study explored diversity of traditional knowledge, collect data on utilization, identify and analyzed the wild edible plants and fungi of Kashmir Himalaya (India). Information was collected from fieldwork by orally consented-structured views with 113 individuals during 2012-2014 from nine rural and mountainous areas. Data were classified according to folklore perceptions (cooked vegetables, salads, spices, chutneys, herbal teas, home-made alcoholic drinks, soups, raw fruits and underground snacks). Information was analyzed using use-reports (UR), use-value (UV), informant consensus (F_{ic}), fidelity level (Fl), and cultural importance index (Cl) metrics. In total, 111 phytotaxa, distributed into 87 genera and 43 families were identified as local edible resources. Overall, 94.25% of angiosperms taxa followed by 3.45% of gymnosperms, and 2.30% of cryptogams traditional used were documented. Several taxa were highly cited (e.g. *Amaranthuscaudatus*, *Angelica archangelica*, *Asparagus racemosus*, *Berberislycium*, *Fragariavesca*, *Hippophaerhamnoides*, *Oxyriadigyna*, *Juglansregia*). Frequently used parts were young leaves (19.82%), followed by parts used as fruits (15.32%), young twigs (9.01%), roots (8.11%), and tubers (6.31%). The most usage mentioned were leafy vegetables (27.93%), followed by uses as raw fruits (22.52%), herbal teas (14.41%), salads (9.91%), alcoholic drinks (6.31%), and underground snacks (4.50%). The study results into high fidelity score and informant consensus factor suggesting that ethnobotanical knowledge could potentially guide the search for developing new neutraceutical products in near future.

2.7 Plant authentication in Janaki Ammal Herbarium

Bikarma Singh, VK Gupta, S. Nanda

During 2015-2016, following bulk quantity identification works were carried out as National Referral Centre for Plant Identification and Authentication:

- 117 Plant identification for Department of Environmental Science, Jammu University
- 87 Plant identification for Department of Botany, Jammu University
- 57 Plant identification for Department of Environmental Science, Central University of Jammu
- 34 Plant identification for CSIR-CDRI, Lucknow

Besides these, small number of plant samples of various other universities/institutes/colleges was carried out such as for GNDU, Bhopal University, SMVDU, Panjabi University, Kashmir University, Sere-Kashmir University, etc.

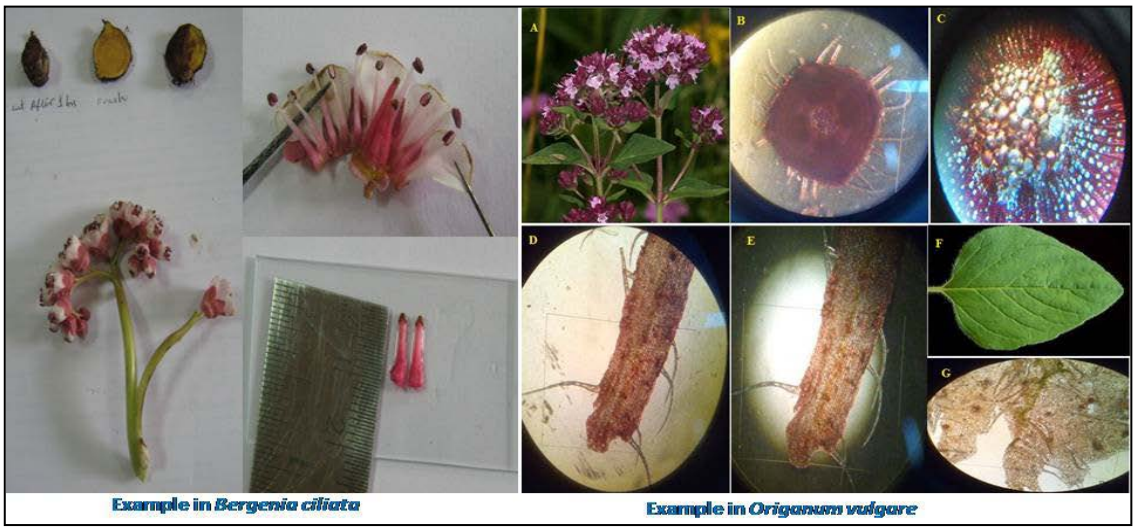


Figure 2.7.1 : Morphological and Anatomical Identification of Herbarium and Crude Drug samples; (a) Dissection of flowers: study on sepals, petals, stamens, ovary, fruits; (b) Anatomical study: transverse/ longitudinal section of different parts like stem, leaves, roots, rhizomes.

2.8 Type III plant polyketide synthases: Enzyme promiscuity, mechanistics and secondary metabolism

Shahzad A. Pandith, Niha Dhar, Ram Vishwakarma and Surrinder K. Lattoo

Plants effectively defend themselves against biotic and abiotic stresses by synthesizing diverse secondary metabolites, including health-protective flavonoids. These display incredible chemical diversity, ubiquitous occurrence and confer impeccable biological and agricultural applications. Chalcone synthase (CHS), a Type III plant polyketide synthase is critical for flavonoid biosynthesis. It catalyzes acyl CoA thioesters to synthesize naringenin chalcone through a polyketidic intermediate. The functional divergence among the evolutionarily generated members of a gene family is pivotal in driving the chemical diversity. Against this backdrop, present study was aimed to functionally characterize members of CHS gene family from *Rheum emodi*, an endangered and endemic high altitude medicinal herb of North-Western Himalayas. It is a rich reservoir of pharmaceutically important secondary metabolite constituents like flavonoids, anthraquinones and stilbenoids. Here, we characterized two full length cDNAs (1179 bp each), *Re*CHS1 and *Re*CHS2 encoding unique paralogs from *R. emodi*.

The highly conserved nature of CHS sequences across species was used to recognize catalytically important residues in *Re*CHS paralogs which showed modest similarity at nucleotide and amino acid levels with each other and with the related orthologous family members. The sequence analysis and homology modelling of the isolated *Re*CHSs revealed that they share similar attributes as found in other known chalcone synthases like the representative Alfalfa CHS2. Additionally, the other identified residues thought to be essential in controlling the substrate and product specificity were also found to show significant level of conservation in the isolated *Re*CHS sequences which in

all suggests that the two paralogs are true CHSs (Figure 2.8.1).

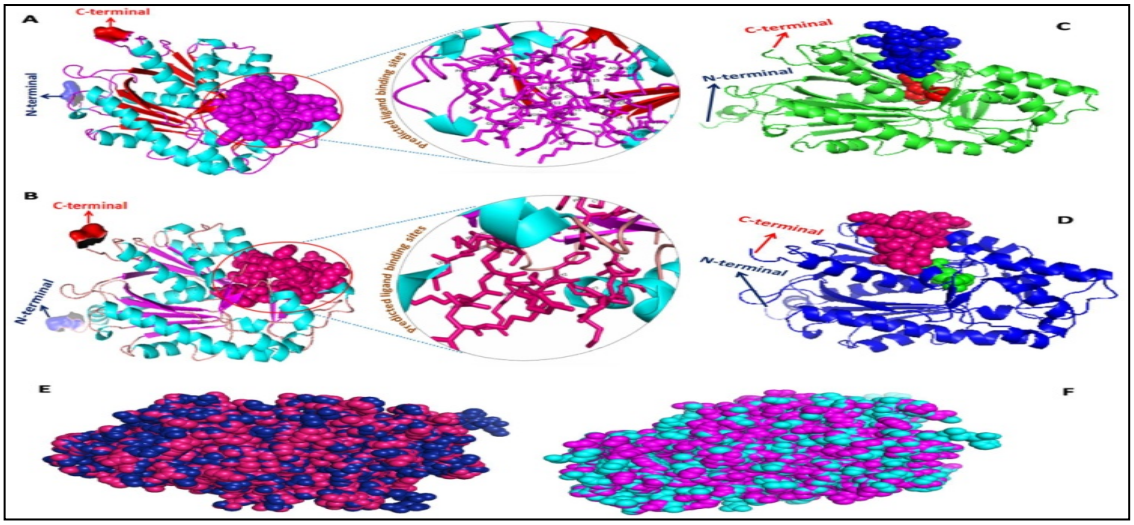


Figure 2.8.1 : Predicted three-dimensional models and ligand-binding sites of *Re*CHSs. (A-B)

Ribbon model display of the three-dimensional structures of *Re*CHS1 (A) and *Re*CHS2 (C) as predicted by Phyre[2] web server, using the crystal structure of *Medicago sativa* CHS (PDB code c1cmla) as template. N- and C-terminal domains are shown as blue and red caps, respectively. The ligand binding sites as predicted by 3DLigandSite web server are depicted in the ribbon model (*Re*CHS1, magenta; *Re*CHS2, pink) and also highlighted as an inset; **(C-D)** Ribbon display of 3D structures of *Re*CHS1 (C) and *Re*CHS2 (D) as predicted by I-TASSER server, using the crystal structure of *Medicago sativa* CHS (PDB code c1cmla) as template. The highly conserved catalytic triad (Cys-His-Asn) is shown in the central core of the structures (*Re*CHS1, pink; *Re*CHS2, violet). The malonyl CoA binding motifs are also depicted (*Re*CHS1, blue; *Re*CHS2, pink); **(E-F)** Superimposition of 3D ribbon models of *Re*CHS1 (E, pink) and *Re*CHS2 (F, cyan) with Alfalfa (blue for *Re*CHS1 and violet for *Re*CHS2) using Pairwise Alignment Tool of FATCAT web server. To elucidate the phylogenetic relationship of deduced primary amino acid sequences of *Re*CHSs with related CHS proteins, phylogenetic analysis was performed. The appearance of *Re*CHS1 and *Re*CHS2 in two distinct and distant clusters points towards their early diverged evolution. Moreover, both *Re*CHS1 and *Re*CHS2 members of *R. emodi* join their respective members from *R. palmatum* in a little clade in two far-away branches. This is indicative that the two CHS variants (CHS1 and CHS2) may have evolved before the speciation event between the two *Rheum* species. In other words, CHS1 and CHS2 possibly diverged in some common ancestor of these two *Rheum* species. The paralogs are distantly related and have evidently diverged in the ancestral lineage of the big clade that they belong to as depicted in the Figure 2.8.2. Further, the equal length of two intronless *Re*CHSs excludes the possible occurrence of duplication event in them. However, our results indicate synonymous/non-synonymous mutation events could have taken place over the period of evolution to generate the two paralogous members of *Re*CHS.

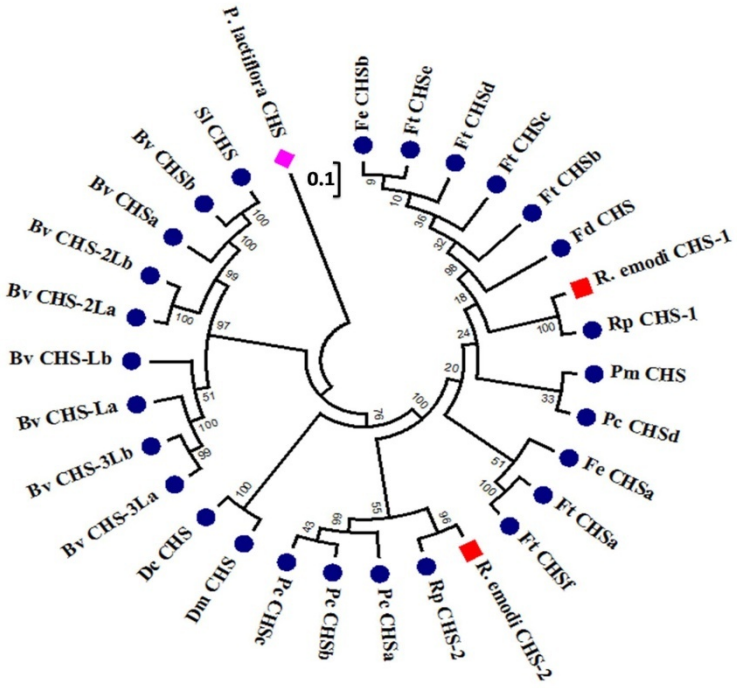


Figure 2.8.2 : Phylogenetic tree of ReCHS1 and ReCHS2.

The phylogenetic analysis was performed using the MUSCLE program and MEGA 6 software based on the neighbor-joining method. The numbers on the nodes indicate the bootstrap values after 1000 replicates. The bar indicates an evolutionary distance of 0.01%. The evolutionary distances were computed using the Poisson correction method. The analysis involved alignment of 30 amino acid sequences which were chosen by scrutinizing the available data related to CHS genes from NCBI data-base at order level (Caryophyllales). About 33 families (as per APG III system, 2009) were screened and desired sequences were selected based on the complete cds information available. The phylogenetic tree was rooted using CHS from Chinese peony (*Paeonia lactiflora*; AEK70334.1) as out-group seeing that it belongs to Saxifragales order (Paeoniaceae family) which shares a close evolutionary relation with Caryophyllales. The numbers on the branches indicate the bootstrap values after 1000 replicates. The most popular and widely used method of proteomics, bottom-up approach was deployed to decipher the identity of *Re*CHS tryptic digests. In general, the sequence coverage in bottom-up proteomic analysis ranges from 5 to 70%. However, we were able to generate a better sequence coverage of *Re*CHS proteins. Moreover, the successful detection and identification of only two unique peptides is generally considered to be sufficient for protein identity. In this study, we have characterised five peptide sequences of *Re*CHS1 and six of *Re*CHS2 (Table 2.8.I). This has further supported the existence of two separate paralogous members of small CHS gene family of *R. emodi*. With the advent of highly sensitive and advanced mass spectrometry analytical tools for proteomic characterisation, it has become possible to complement the conventional western blot techniques requiring antibody generation.

Table 2.8.I: List of fragment ions generated in the mass spectrum and tandem mass spectrum related to the peptide identification from the tryptic digests of *Re*CHS1 and *Re*CHS2 proteins.

Protein	Retention time min)	Precursor ion	Theoretical mass (*m/z*)	Experimental mass (*m/z*)	Peptide sequence
***Re*CHS1**	12.42	630.7983$[M+2H]^{+2}$	1259.5822	1259.5816	VTNSDHMTDLK
	15.46	580.2929$[M+2H]^{+2}$	1158.5708	1158.5703	QDMVVSEVPR
	16.33	828.4812$[M+H]^{+1}$	827.4737	827.4753	DVPGLISK
	17.38	847.4048$[M+3H]^{+3}$ 1270.600$[M+2H]^{+2}$	2537.1835	2537.1964	ITHVIMCTTSGVDMPGADYQLTK
	18.24	421.5535$[M+3H]^{+3}$ 631.8258$[M+2H]^{+2}$	1261.6366	1261.6377	YMHLTEDLLK
***Re*CHS2**	5.61	553.2109$[M+H]^{+1}$ 575.1909$[M+Na]^{+1}$	552.2030	552.2036	MCDK
	13.12	430.3021$[M+H]^{+1}$	429.2944	429.2951	LGLK
	13.51	607.309$[M+H]^{+1}$	606.3044	606.3047	SMIEK
	15.46	491.8245$[M+2H]^{+2}$ 982.6379$[M+H]^{+1}$	981.6338	981.6335	LLGLRPSVK
	18.55	482.2695$[M+2H]^{+2}$ 963.5290$[M+H]^{+1}$	962.5235	962.5185	LGSPFISSR
	18.96	933.9299$[M+2H]^{+2}$	1864.8413	1864.8423	FMMYQQGCFAGGTVLR

The kinetic characterization of *Re*CHSs demonstrated that the active site of *Re*CHS2 seems more flexible to non-physiological substrates as evident from its catalytic efficiency (Table 2.8.II). Further, *Re*CHS1 was found to show a broad range of pH stability compared to that of *Re*CHS2 which was found to be active over a limited range of pH variance (Figure 2.8.3).

Table 2.8.II: Steady state kinetic parameters of purified *Re*CHSs with different starter units

Starter CoA	*Re*CHS1			*Re*CHS2		
	K_m (µM)	**V_{max} (pmol $min^{-1}mg^{-1}$)**	**Efficiency (V_{max}/ K_m)**	**K_m (µM)**	**V_{max} (pmol $min^{-1}mg^{-1}$)**	**Efficiency (V_{max}/ K_m)**
p-Coumaroyl-CoA	38.43	42	1.092	155.8	37.0	0.237
Acetyl-CoA	46.6	3.23	0.069	37.3	63.5	1.702
Butyryl-CoA	13.9	1.0	0.071	8.01	11.0	1.373
Hexanoyl-CoA	91.65	1.27	0.013	11.4	4.7	0.412
Octanoyl CoA	22.0	1.1	0.050	26.6	21.4	0.804
***Results are means (n = 3) with SD values below 10% in all the cases**						

Phylogenetic analysis revealed the existence of higher rate of synonymous substitutions in the intron-less divergents of *Re*CHS. Furthermore, *Re*CHS2 displayed a mutation in the cyclization pocket of active site domain wherein Ile^{255} and Gly^{257} were non-synonymously replaced by Leu and Ala. Amino acid replacement rates are often examined in conjunction with shifts in enzyme function. *Re*CHS2 exhibited higher activity with non-physiological substrates also when compared to that of *Re*CHS1. Additionally, it also displayed significant enzymatic efficiency (V_{max} / K_m) with different substrates.

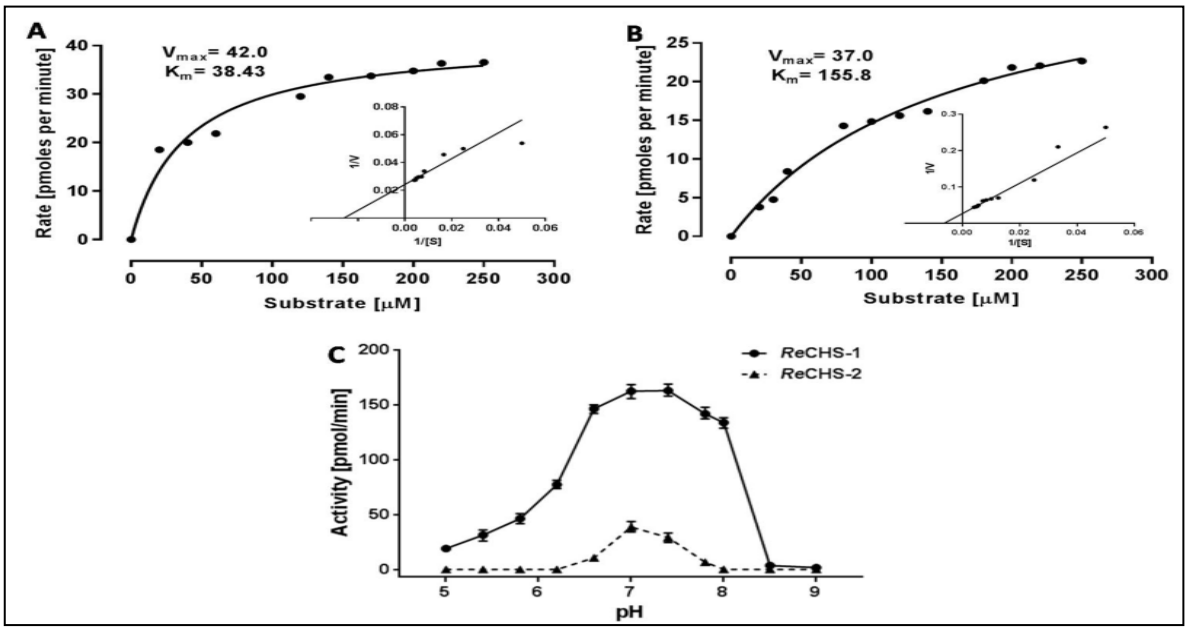


Figure 2.8.3 : ***Kinetic study of ReCHSs: (A-B);***

Michaelis-Menten plots of *Re*CHS1 (A) and *Re*CHS2 (B) with an inset Lineweaver-Burk plot. The kinetic parameters K_m and V_{max} were calculated by nonlinear regression analysis using GraphPad Prism 6 software. **(C)**; The activity of *Re*CHSs were assayed at varied pH concentrations (pH = 5.0 to 9.0). Citrate buffer, potassium phosphate buffer, and 0.1 M Tris/HCl buffers were used for pH= 3.0-6.2, 5.8-8.0 and 8.5-9.0, respectively. The CoA esters (p-coumaroyl-CoA and malonyl-CoA) were used as substrates and the production of naringenin/naringenin chalcone was quantified as activity (pmol/min). Values are the means ± standard deviation of at least three replicates. Points of variance (n=3) are depicted at each point in section C of the graph. The differences in the relative activity and enzyme efficiency as displayed by *Re*CHS paralogs could be a manifestation of the variation in their cyclization pocket. There were significant spatial and altitudinal variations in mRNA transcript levels of *Re*CHSs correlating positively with metabolite accumulation. Additionally, exogenous (MeJ, SA and UV-B) and endogenous (wounding) elicitations, chosen on the basis of identified *cis*-regulatory promoter elements, presented considerable differences in the transcript profiles of *Re*CHSs. We observed differential propensity of *Re*CHS paralogs in terms of accumulation of flavonoids / anthraquinones and their relative substrate selectivities. Taken together, our investigations establish that *Re*CHS1 paralog displays prime involvement in flavonoid biosynthesis, while as *Re*CHS2 seems more flexible towards substrate selectivity and may be implicated in the biosynthesis of polyketidic anthraquinones. The homodimeric Type III

PKSs seem to display substantial substrate promiscuity and metabolic diversity. These divergent paralogs are suitable for production of novel polyketide scaffolds with promising biological activities employing protein engineering.

2.9 De Novo transcriptome analysis reveals putative pathway genes involved in biosynthesis and regulation of camptothecin in Nothapodytes nimmoniana (Graham) Mabb.

Gulzar A. Rather, Shahzad A. Pandith, Arti Sharma, Prashant Misra, Surrinder K. Lattoo

Nothapodytes nimmoniana (Graham) is a medium sized tree species found in Western Ghats of India which represents a biodiversity hotspot. The tree is the richest source of a potent anti-cancer monoterpene indole alkaloid (MIA) camptothecin (CPT) & 9-methoxy camptothecin. CPT, a water insoluble MIA. It has been considered as one of the most promising anticancer drugs of 21st century. It exhibits antitumor activity by inhibiting DNA topoisomerase I. Despite tremendous importance of CPT, its biosynthesis remains largely unresolved. The initial step of CPT biosynthesis involves strictosidine synthase (STR) mediated condensation of tryptamine with the iridoid glucoside secologanin to yield strictosidine (Figure 2.9.1). Subsequently, intramolecular cyclization of strictosidine yields strictosamide, a penultimate precursor for biosynthesis of camptothecin. In addition, the enzymatic steps between strictosamide & camptothecin possibly involve cytochrome P450- dependent reactions.

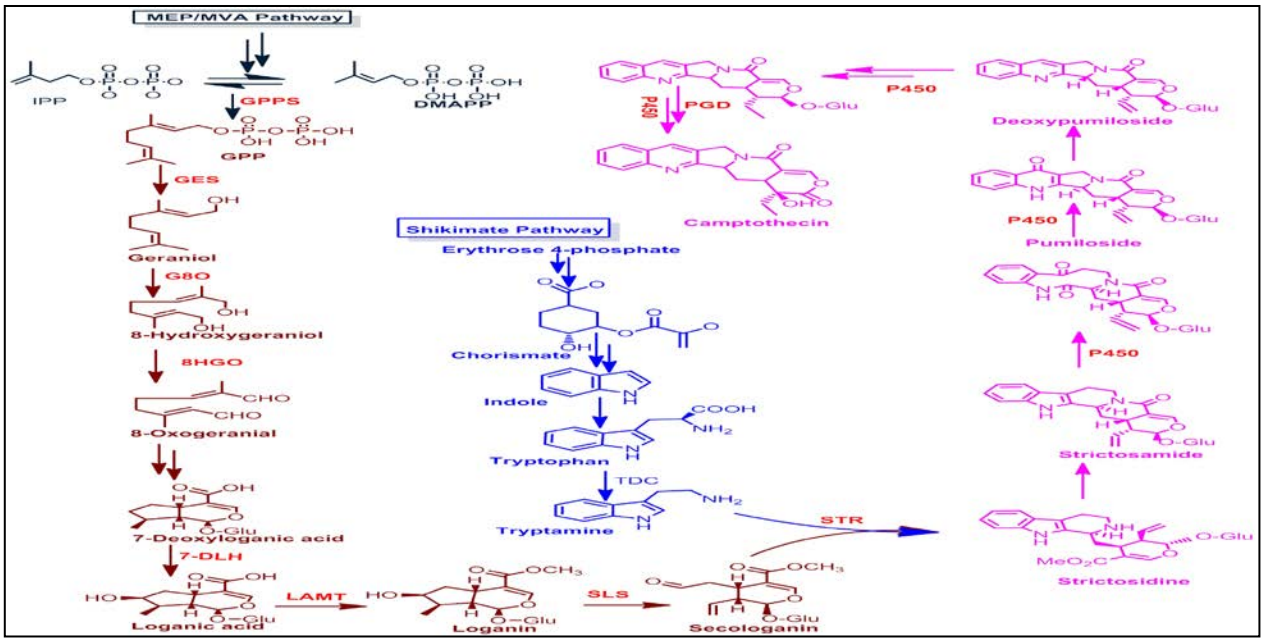


Figure 2.9.1 Putative biosynthetic pathway of CPTGPPS= geraniol pyrophosphate synthase; GES= geraniol synthase; G8O= geraniol-8-oxidase; 8-HGO= 8-hydroxy geraniol oxidoreductase; 7-DLH= 7-deoxyloganic acid hydroxylase; LAMT= loganic acid methyltransferase; TDC= tryptophan decarboxylase; SCS=secologanin synthase; STR= strictosidine synthase; PGD= putative strictosidine β-D glucosidase.

The knowledge regarding the biosynthetic machinery for CPT remainsis yet to be fully deciphered due to lack of molecular, genetic and genomic resources. The advent of deep sequencing technologies (NGS- next generation sequencing) allows to deliver large amount of sequence information complemented by bioinformatic approaches. These strategies involving genomic and/or transcriptomic analysis have enabled the discovery of new genes vis-à-vis biosynthetic elucidation and regulation of different metabolic pathways. Phytochemical analysis of different tissues of *N. nimmoniana* harvested at nine different phenophases showed interesting differences in the content of CPT. In general, CPT content was detected in low concentrations at early phenophases which gradually increased towards the later stages. The phenophasic variation in the CPT content of different tissues ensured their harvesting at proper stage for transcriptome analysis. The leaf and root wood tissues selected for transcriptomic analysis were harvested at fruit set stage (Figure 2.9.2).

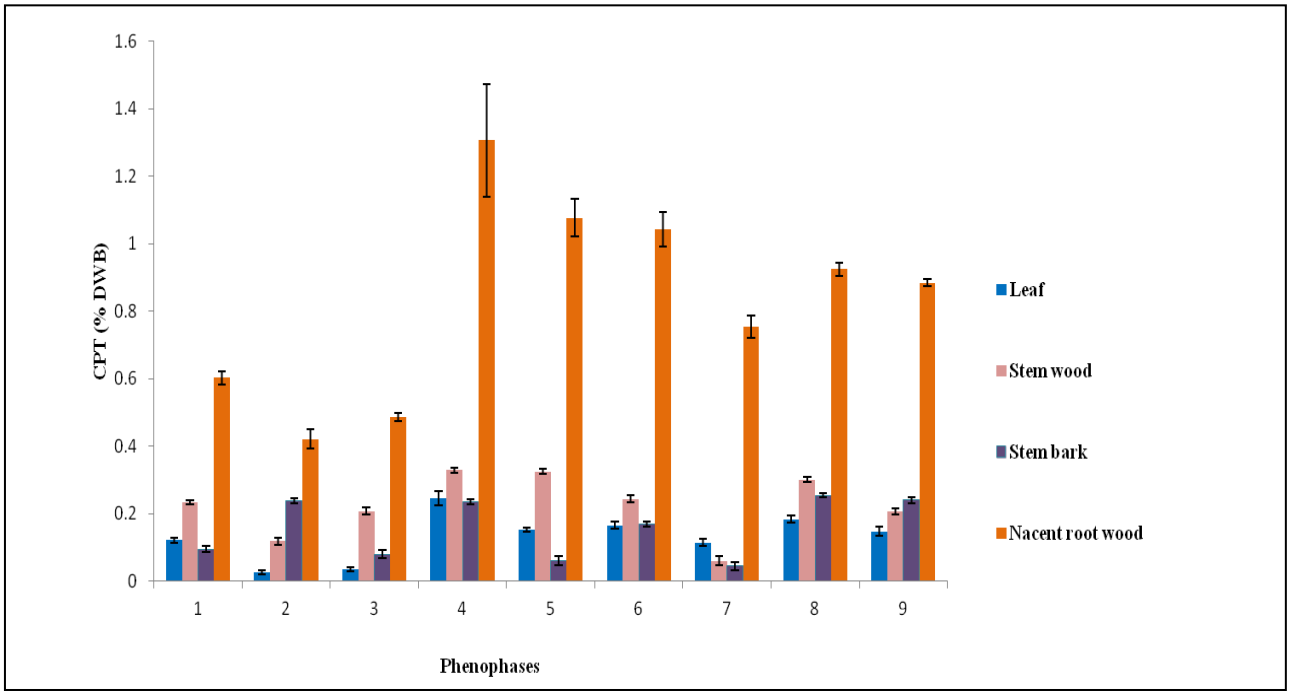


Figure 2.9.2 : Chemoprofiling at different ontogenic stages of *Nothapodytes nimmoniana*. 1= vegetative phase; 2= inflorescence emergence; 3= flowering; 4= fruit-set; 5= young fruit; 6= fruit-maturation; 7= over maturation; 8= fruit drop; 9= senescence.

N. nimmoniana transcriptome was generated by subjecting leaf and root wood tissues to next generation sequencing using Illumina platform. Assembly of the raw reads obtained from cDNA libraries resulted in the generation of 31172889 and 31218626 reads from leaf and root wood tissues, respectively. The Gene Ontology (GO) classification system was used to describe the possible functions of genes and associated gene products. The number of transcript contigs under biological process were 3815 while as 2376 transcript contigs grouped under molecular function. The cellular component clustered 1256 contigs (Figure 2.9.3).

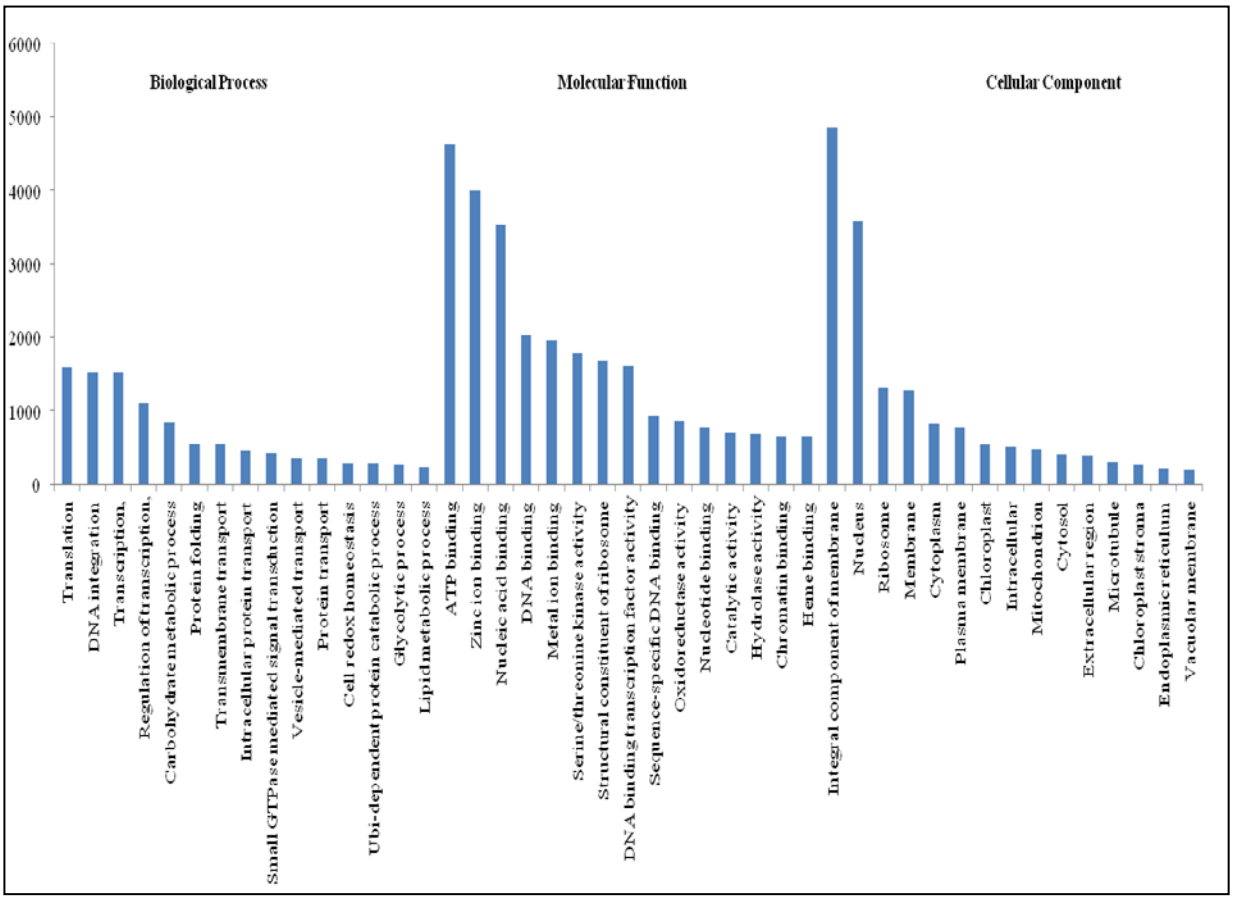


Figure 2.9.3: Gene Ontology (GO) classification. Bar chart representing functional categorization of unigene transcripts annotated from comparative RNA-Seq data of *N. nimmoniana*. The results are summarized in three main categories, Biological process, Cellular component, and Molecular function.

CPT biosynthesis is a very complex process involving many distinct enzymatic steps. In *N. nimmoniana* transcriptome dataset multiple transcripts encoding almost all known enzymes involved in MVA, MEP and up-stream steps of CPT biosynthesis pathway were identified (Table 2.9.1). Furthermore, we identified putative cytochrome P450 transcripts which belong to 46 cytochrome P450 sub-families, according to the standard CYP family categories. Additionally, a total of 16 cytochrome P450 transcripts involved in secondary metabolism were identified as candidate genes for further characterization

.

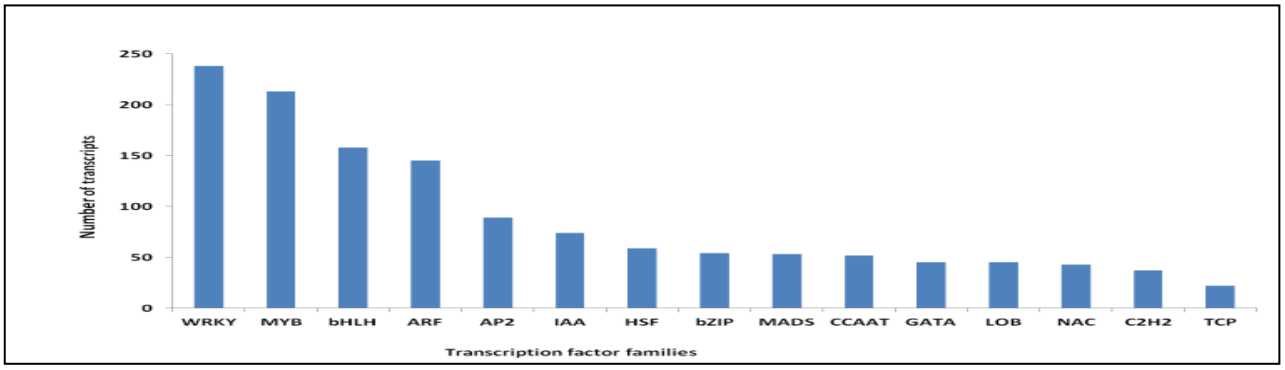
Table 1. Identified genes involved in camptothecin biosynthesis along with their FPKM (fragments per kilo base of transcript per million mapped reads) values.

Annotation	Contig ID	Leaf read count	Leaf FPKM	Root read count	Root - FPKM
Acetyl CoA –acetyltransferase	c59752_g1_i4	464	25.61490664	224	5.073424908
HMG CoA synthase	c63405_g1_i1	432	12.2831674	1323	21.71503318
HMG CoA reductase	c62568_g1_i6	028	1.655575753	873	13.79540474
mevalonate kinase	c52448_g1_i2	160	4.340928438	445	9.343982212
phosphomevalonate kinase	c58210_g1_i2	938	15.06338077	607	10.39684522

diphosphomevalonate decarboxylase	c57408_g1_i3	376	8.800483722	1901	34.13807218
1-deoxy-D-xylulose-5-phosphate-	c57121_g2_	743	23.73285569	225	4.20684612
Synthase 1-deoxy-D-xylulose-5-phosphate-	c63870_g2_i3	707	27.7281067	2904	55.19223454
reductoisomerase 2-C-methyl-D-erythritol 4-phosphate-	c51698_g1_i1	602	15.07998086	198	4.640982856
Cytidylyltransferase 4-diphosphocytidyl-2-C-methyl-D-	c56974_g1_i1	110	4.99855934	534	10.86613755
erythritol kinase 2-C-methyl-D-erythritol 2,4-	c58761_g1_i1	1985	65.68634541	1571	52.50638026
cyclodiphosphate synthase 4-hydroxy-3-methylbut-2-enyl-	c57052_g2_i1	2759	157.2786355	1935	32.60635433
diphosphate reductase isopentenyl-diphosphate isomerase	c62948_g1_i1	417	50.07507089	915	44.04630676
geranyl diphosphate synthase	c54310_g2_i2	92	8.482563592	10	1.017428609
geraniol synthase	c56678_g1_i2	0	0	687	11.81
geraniol-10-hydroxylase	c39167_g1_i5	0	0	6152	116.9223922
geraniol-8-hydroxylase	c64011_g1_i1	714	26.26353093	274	8.048434804
8-hydroxygeraniol oxidoreductase	c57797_g1_i5	0	0	1745	33.32184113
Iridoid synthase	c61987_g1_i1	50205	1685.290407	2607	79.92607737
7-deoxyloganetic acid	c53935_g1_i1	779	15.69881499	1000	17.89816144
7-deoxyloganic acid hydroxylase	c58851_g1_i3	33	0.610464605	1680	23.64056633
Loganic acid o-methyltransferase	c51527_g1_i3	0	0	541	20.31268384
Secologanin synthase	c54487_g1_i1	16	2.781649666	6073	100.2915274
Strictosidine synthase	c55350_g1_i3	47	4.574903272	441	11.63939994
Strictosidine glycosidase	c61619_g1_i5	1376	66.41380681	154	3.092704225

Additionally 1683 putative transcription factors (TFs) were identified from the transcriptomic data pool distributed in at least 53 families. It was interesting to note that TFs belonging to WRKY family were found to be most abundant (14.14%), followed by MYB (12.66%) and AP2 (5.29%) as depicted in Figure 2.9.4.

Figure 2.9.4: Identification of transcription factors (TFs). Bar chart representing the distribution of *N. nimmoniana* unigenes in different transcription factor families



The phylogenetic analysis was carried out using three known CPT pathway CYPs [Secologanin synthase (SLS), 7-deoxyloganic acid hydroxylase (7DLAH) and geraniol10-hydroxylase (G10H)] and three putative CYPP450 unigene transcripts (Cyp76A26, Cyp71D11 and Cyp72A219). The proteins corresponding to these genes as well as their close homologs from other plants grouped into two major phylogenetic clusters. G10H, CYP76A and CYP71D formed one cluster whereas SLS, 7-DLAH and CYP72A formed a separate cluster. Furthermore, G10H and CYP76A shared a closer relation with each other compared to that of CYP71D. In a similar fashion, SLS and 7DLAH exhibited more relatedness to each other than to their other member CYP72A. In either of the clusters, G10H and CYP76A and SLS and 7DLAH shared a common but separate recent ancestor. From these results, CYP72A219, CYP71D11 and CYP76A26 unigenes likely seem to have possible involvement in downstream CPT biosynthetic pathway (Figure 2.9.5).

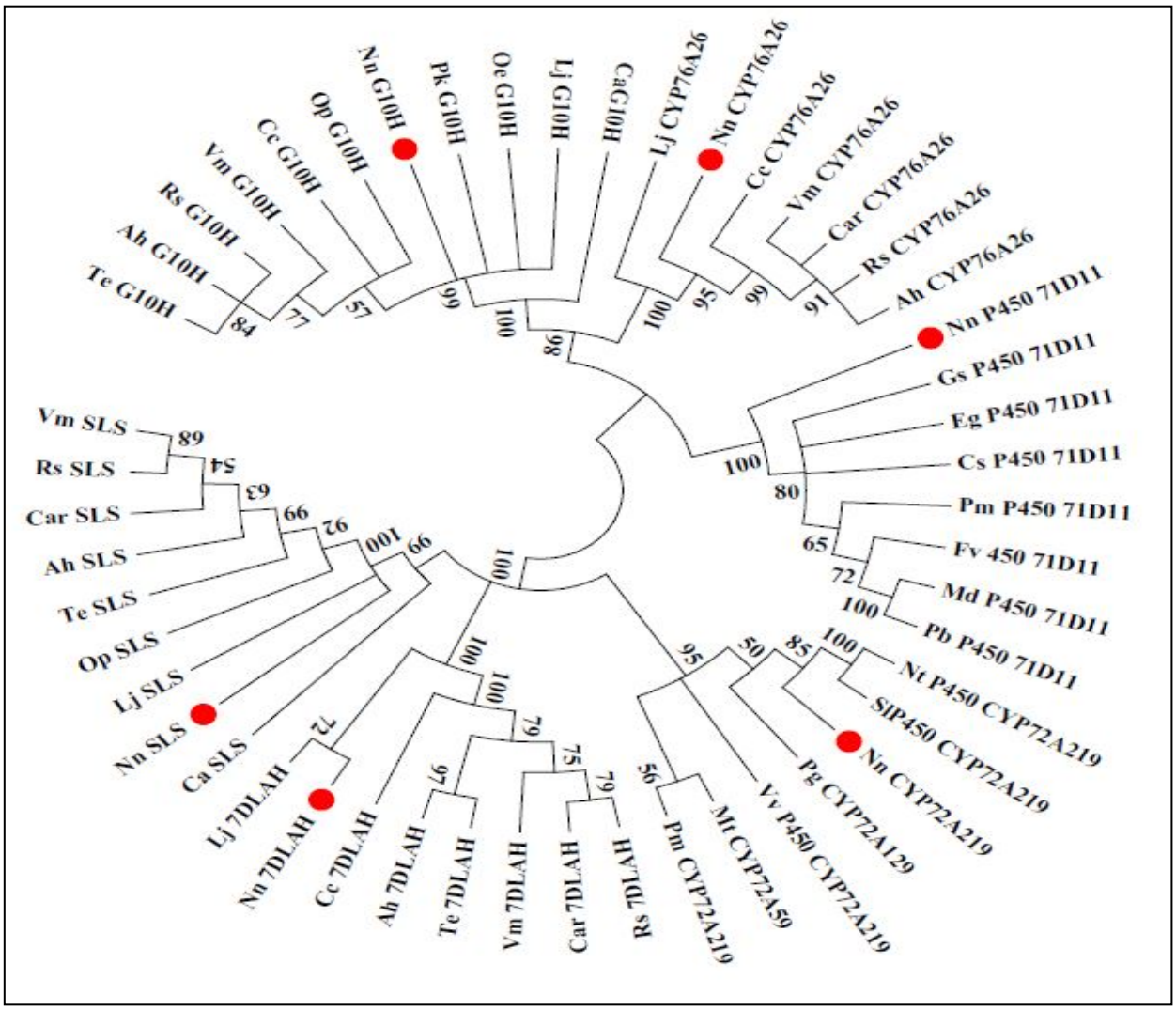


Figure 2.9.5: Phylogenetic analysis of CYPs. MUSCLE program of MEGA 6.06 software was used to construct the evolutionary tree by aligning amino acid sequences of 57 CYP genes belonging to 24 plant species. The evolutionary distances were calculated with Poisson correction method. The numbers on the nodes indicate the bootstrap values after 1000 replicates.

Differential gene expression (DEG) profiles can be employed to identify transcripts involved in tissue-specific accumulation of specialized metabolites. The DEG data obtained from transcriptomic analysis of leaf and root wood tissues of *N. nimmoniana* was further validated by qRT-PCR analysis of 14 unigenes/TFs. These were selected based on their role in CPT biosynthesis. Most of the transcripts were found to be up-regulated in root wood and down regulated in leaf tissue (Figure 2.9.6). In general, the relative mRNA transcript level of the selected genes/TFs corroborated well with that of the RNA-Seq results. To sum up, we have developed a transcriptome resource of *N. nimmoniana* that led to the identification of large data set of unigenes including known and putative candidate genes of CPT biosynthesis pathway. Characterization of identified TFs and several P450s could pave way for unravelling the pathway and regulatory aspects of CPT biosynthesis vis-à-vis its higher production in homo- and/or hetero-logous host systems.

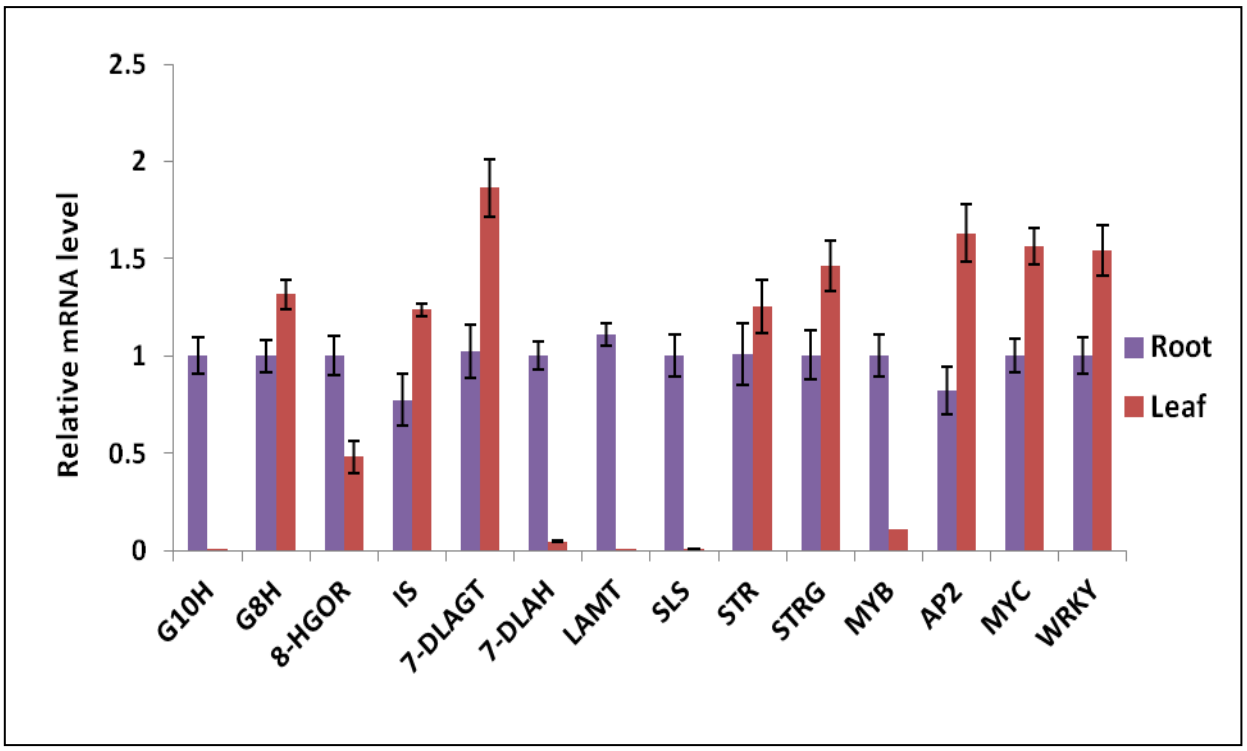


Figure 2.9.6: qRT-PCR validation of differentially expressed genes.
G10H= Geraniol 10-hydroxylase, G8H= Geraniol 8- hydroxylase; 8-HGOR= 8- hydroxygeraniol oxidoreductase; 736 IS= Iridoid synthase; 7-DLAGT=deoxyloganic acid glycosyltransferase; 7-DLAH= 7-deoxyloganic acid hydroxylase; LAMT=Loganic acid methyl transferase; SLS= Secologanin synthase; STR= Strictosidine synthase; SGD= Strictosidine β-D-glucosidase; MYB, AP2, MYC and WRKY transcription factors.

2.10 Molecular characterization of DWF1 from *Withania somnifera* (L.) Dunal: its implications in withanolide biosynthesis.

Sumeer Razdan, Wajid Waheed Bhat,, Surrinder K. Lattoo

Withania somnifera (L.) Dunal (Solanaceae) is a highly reputed medicinal plant used in Ayurveda since antiquity. It is being used as an indispensable component of several medicinal formulations for the treatment of various ailments and diseases. Through chemical investigations, it has been revealed that *W. somnifera* synthesizes an array of secondary metabolites such as alkaloids, flavonoids, tannins and steroidal lactones which show wide spectrum of pharmacological properties. Most of these biological properties have been attributed to a group of steroidal lactones known as withanolides. Although, *W. somnifera* has been investigated thoroughly in terms of its chemical profile but there exists fragmentary information regarding the pathway genes and enzymes involved in withanolide biosynthesis (Figure 2.10.1).

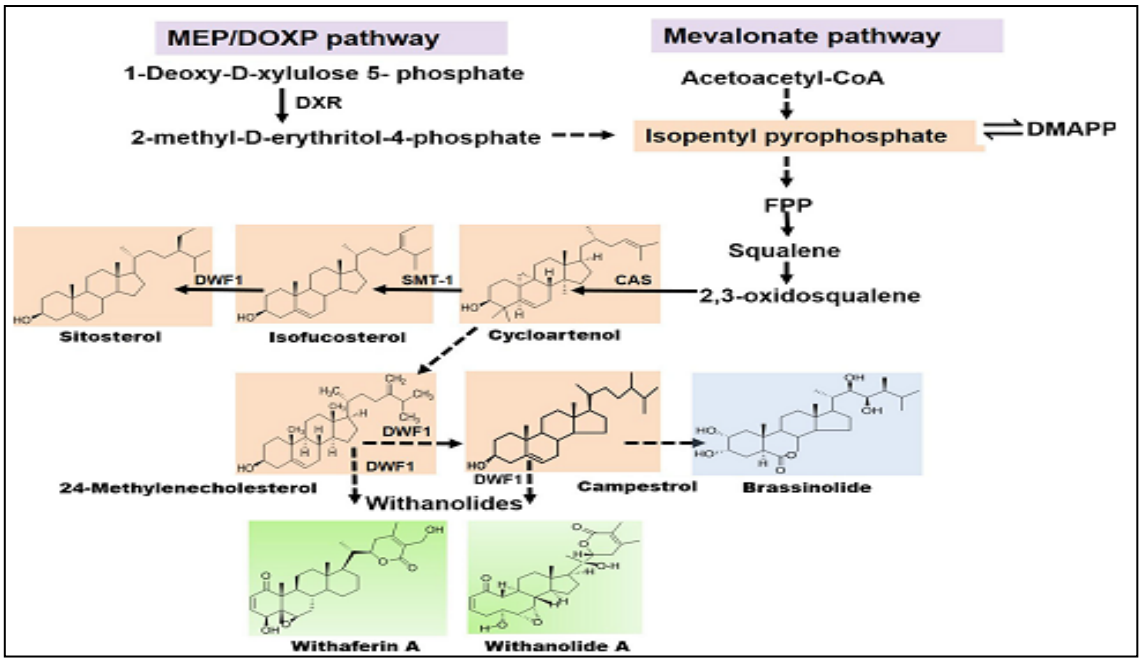


Figure 2.10.1: An overview of putative withanolide biosynthetic pathway. DXR1= Deoxy D-xylulose5-phosphate reductoisomerase, HMGR= 3-hydroxy-3-methyl-glutaryl CoA reductase, IPP= isopentenyl pyrophosphate, DMAPP= dimethylallyl pyrophosphate, IPP= isopentenylpyrophosphate, SMT-1= sterol methyl transferase, DWF1 dimunto/DWARF1. Dashed lines represent multiple enzymatic steps.

Against this backdrop, present study was carried out which entails isolation and molecular characterization of DWF1 from *W. somnifera*. The *Ws*DWF1 was cloned in *E. coli* and the obtained sequence information was confirmed using various *in silico* analysis tools (Figure 2.10.2 and Figure 2.10.3). The cds region was further heterologously expressed in bacterial host (*E. coli*) using pGEX-4T2 vector expression system (Figure 2.10.4). The study also includes deciphering the role of different elicitors in regulation of *Ws*DWF1 in relation to withanolide accumulation.

Figure 2.10.2 : Nucleotide and the deduced amino acid sequence of WsDWF1from *W. somnifera*. The ATG start codon at position 50 bp and the TGA stop codon at position 1649 are highlighted 5 and 3 UTR are in italic.

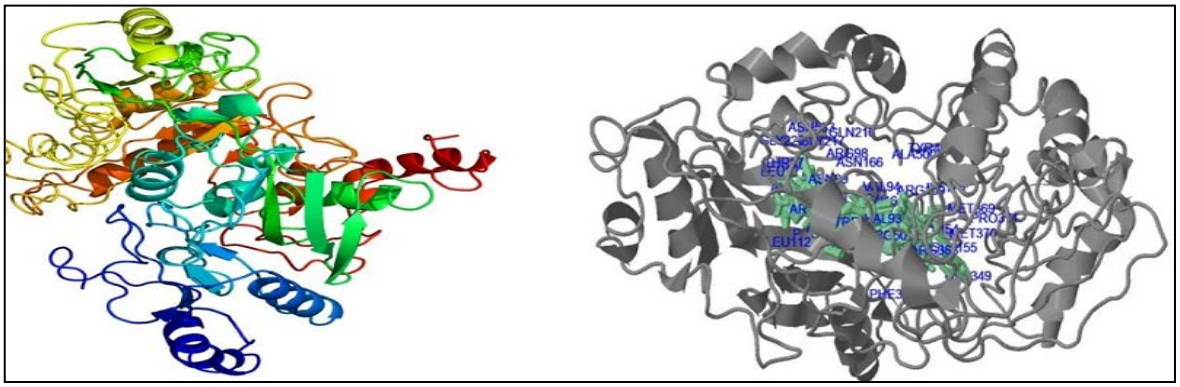


Figure 2.10.3: Prediction of three dimensional structure of *WsDWF1* using Phyre2 bioinformatics tool. *a)* Predicted structure of*WsDWF1* was based upon 6 protein templates (C4ml8C, C4fdoA, C4bc9C, C3bw7A, C3rjaA, C2bvfA). *b)* Prediction of ligand binding sites using 3D ligand site web server. *ADP*, *ZEA* and *FAD* heterogens, ligand binding interactions with the predicted binding sites are shown in the picture.

The regulatory implications of *Ws*DWF1 in withanolide biosynthesis may provide a new possibility for pathway modulation as it is also a pivotal gene involved in brassinolide biosynthesis. It converts 24-methylenecholestrol to campesterol and isofucosterol to sitosterol. The conversion process involves the initial isomerization and subsequent reduction of D24 (28) bond. Mutant studies have shown that DWF1 protein is important for converting 24-methylenecholestrol to campesterol, a main precursor for brassinolide biosynthesis. Studies have also shown 24-methylenecholestrol as a common precursor for formation of withanolides.

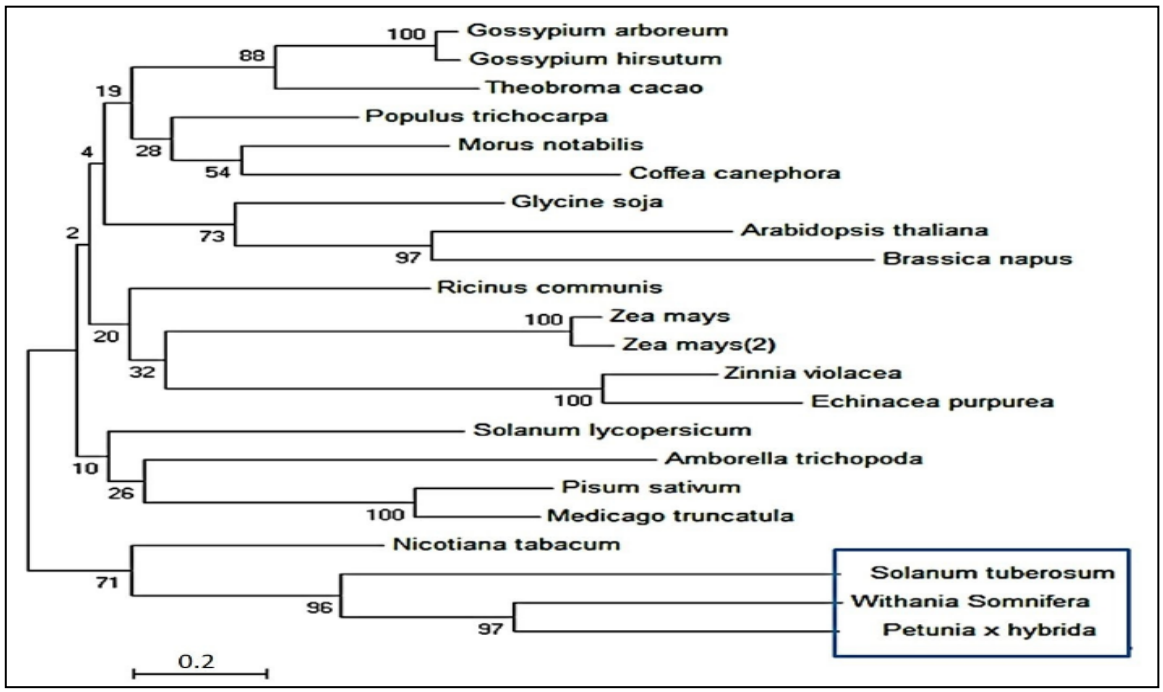


Figure 2.10.4 Heterologous protein expression in *E. coli* with IPTG induction (1 mM) at 37 ˚C for 2, 4, 6 h. Lane 1: Protein molecular weight marker (MW), Lane 2: Whole cell lysate of Uninduced *Ws*DWF1. Lane 3: *E. coli* harbouring empty vector (pGEX-4T-2). Lane 4–6: *E. coli* harbouring pGEX-*Ws*DWF1construct induced by IPTG (1 mM) at 2, 4, 6 h, respectively.

Using the MEGA 5.05 tool a phylogenetic tree was constructed to identify the evolutionary relatedness among different plant species. There was a correlation between the amino acid similarity and degree of relatedness among various plant species predicted in the evolutionary tree (Figure 2.10.5).

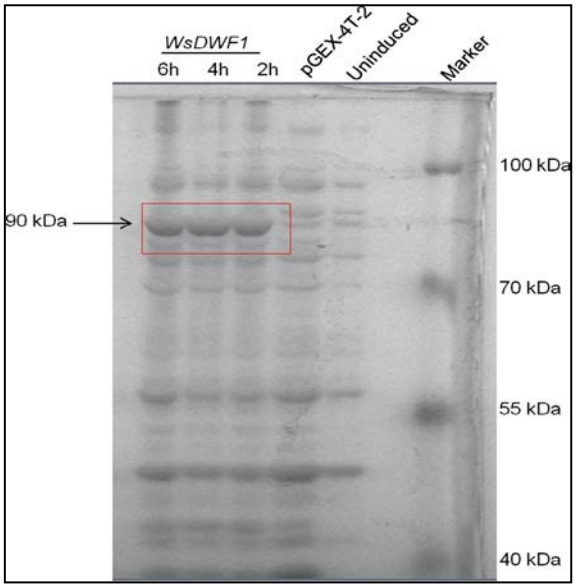


Figure 2.10.5: Neighbour joining phylogenetic tree constructed from the deduced amino acid sequences of various organisms using MEGA 5.05. Numbers above the branches indicate bootstrap values. Members of the Solanaceae family including *W. somnifera* are present in a separate clade

Quantitative RT-PCR analysis was performed to ascertain the comparative expression profile of *Ws*DWF1transcripts in the tissues of roots, stem and leaves of *W. somnifera*. Results demonstrated highest accumulation of *Ws*DWF1transcripts in leaves followed by stalk and least in roots. High expression of *Ws*DWF1in leaf tissue as compared to root tissue is coincident with the higher amount of withanolides in leaf tissue in comparison to root tissue (Figure 2.10.6).

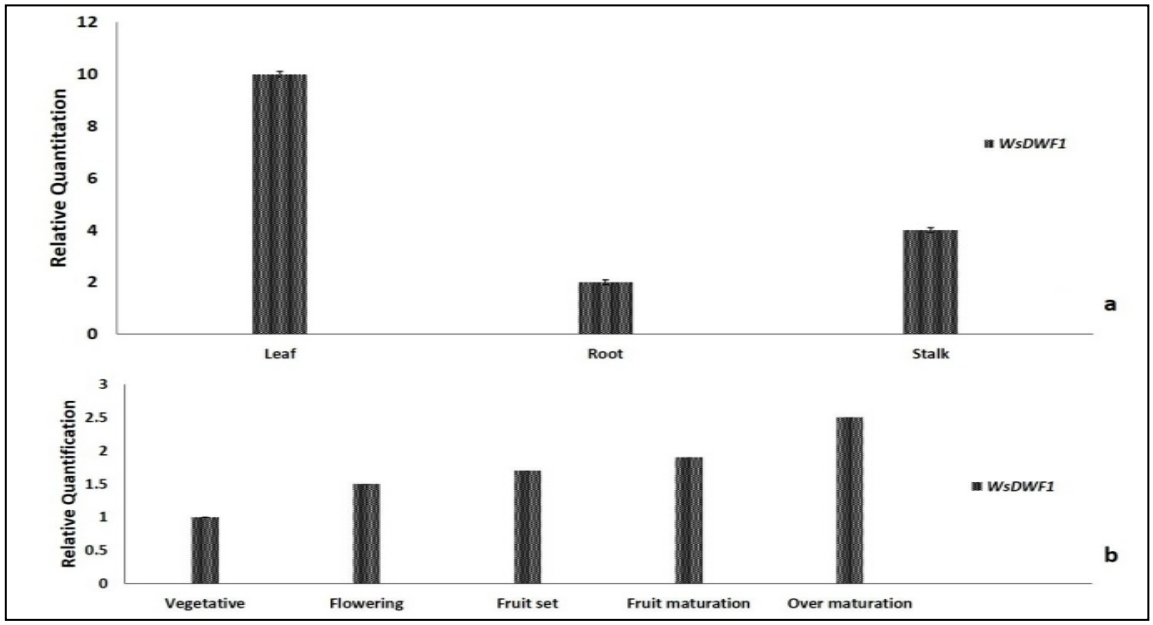


Figure 2.10.6: Tissue-specific real-time expression analysis. Quantitative estimation of the expression of *WsDWF1* in leaf, roots and stalk of *Withania somnifera.* Values are means, with standard errors indicated by bars, representing three independent biological samples, each with three technical replicates. Differences were scored as statistical significance at $*p < 0.05$ and $**p < 0.01$ levels. S2. (B) Transcript profiles of *WsDWF1* during various ontogenetic stages of *Withania somnifera*. a) vegetative, b) flowering c) fruit set d) fruit maturation e) over maturation stages

The focus of present study has been to understand the nature of *Ws*DWF1 regarding the withanolide biosynthesis. The yeast extract (YE) elicitation increased the *Ws*DWF1 transcript levels and corresponding withanolide levels (Figure 2.10.7). These results tend to be in agreement with our earlier studies wherein the use of YE as a repressor of metabolic branch-point junction involving oxidosqualene cyclases (OSCs) resulted in the diversion of metabolic flux towards withanolide biosynthesis. A correlation was found between the increased transcript levels and withanolide A (WS-1) and Withaferin A (WS-3) accumulation upon elicitor treatment with the methyl jasmonate (MeJA) (Figure 2.10.8) and salicylic acid (SA) (Figure 2.10.9).

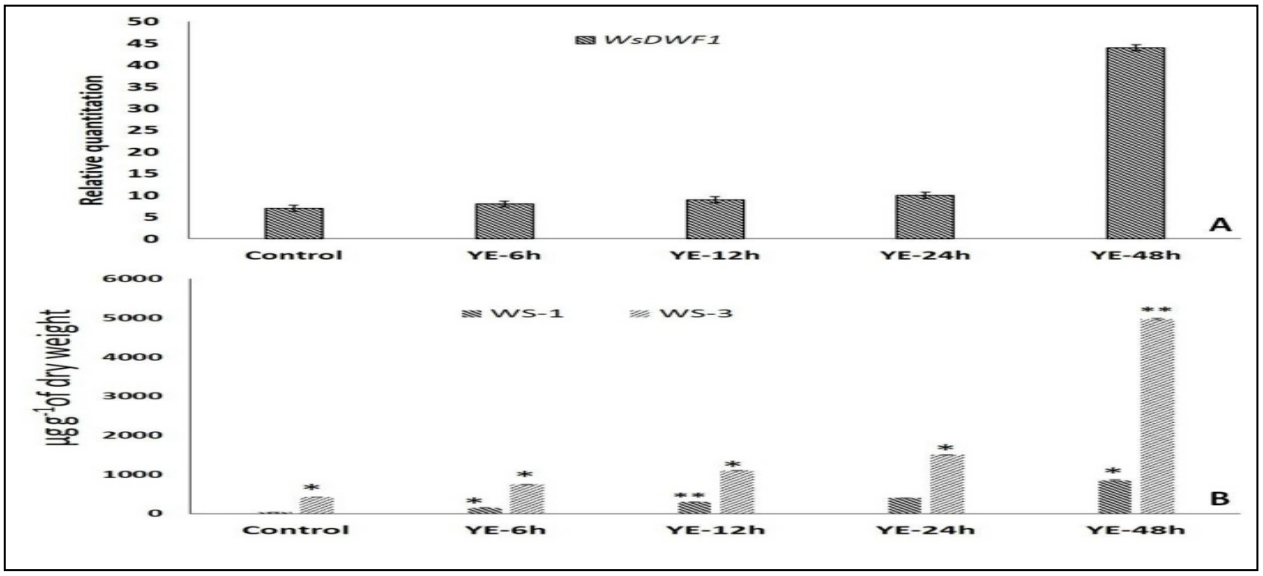


Figure 2.10.7: Time courses of *WsDWF1* expression in micro propagated *Withania somnifera*. The elicitation was done by yeast extract (*YE*; 0.1 % w/v). Experiments were performed in triplicate with similar results; error bars indicate ± standard deviation of the mean. (B) 0.1 %w/v yeast extract (*YE*) at different time courses. Variation in two key withanolides - withanolide A (*WS-1*) and Withaferin A (*WS-3*) was confirmed by HPLC analysis at 6, 12, 24 and 48 h. All values obtained were means of triplicate with standard errors. Time-course accumulation of *WS-1* and WS-3 was statistically significant at $*p < 0.01$level and $**p < 0.01$ levels.

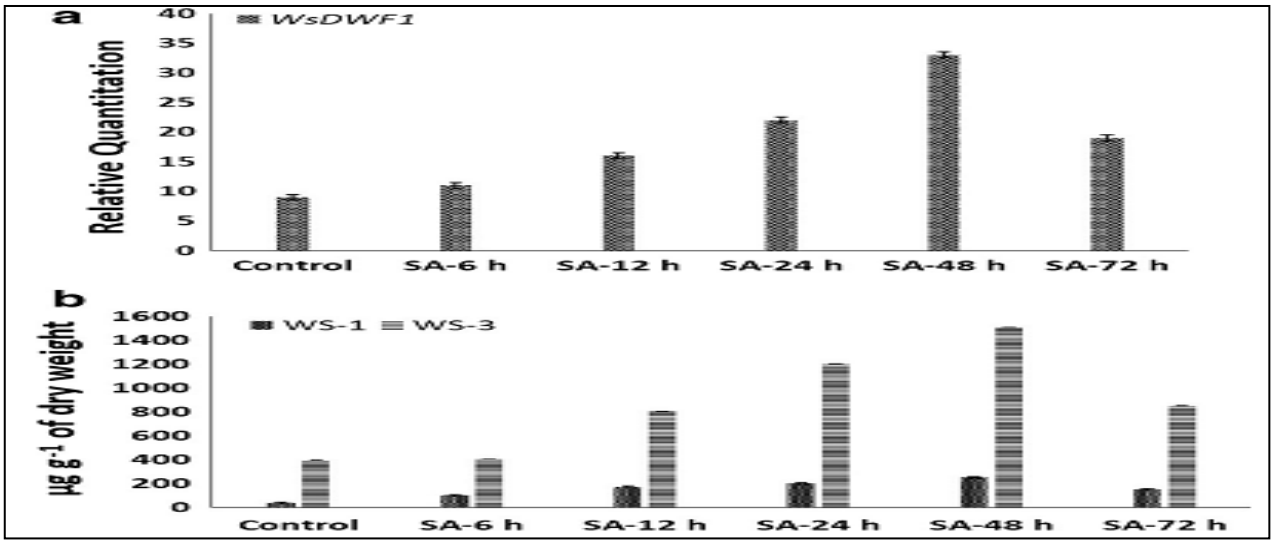


Figure 2.10.8: *a)* Transcript profiles of *Ws*DWF1in response to elicitor treatments. Time courses of *Ws*DWF1expression in micro propagated *Withania somnifera* elicited by methyl jasmonate (MeJA; 0.1 mM). *b)* Time course effect of elicitor treatments on withanolides accumulation. Effect of methyl jasmonate (MeJA) treatment on withanolides accumulation at different time intervals. HPLC analysis demonstrated the change in two key withanolides of withanolide A (WS-1), and Withaferin A (WS-3) at 6, 12, 24, 48 and 72 h after treatments of micro-shoots with 0.1 mM MeJA.WS-3was observed to be enhanced more with respect to WS-1. All values obtained were means of triplicate with standard errors.

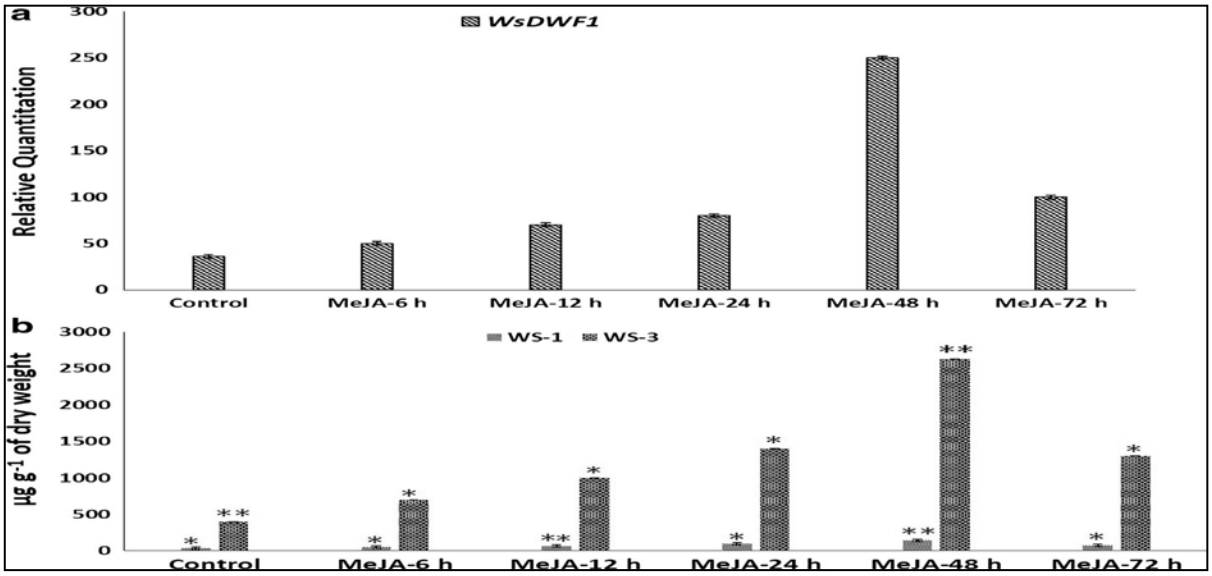


Figure 2.10.9: *a)* Time courses ofWsDWF1expression in micro propagated Withania somnifera elicited by Salicylic acid (SA; 1 mM). Increase in the transcript levels of WsDWF1 was found with the increasing time phase, with highest expression observed at 48 h. *b)* Effect of salicylic acid (SA) on withanolide accumulation at different time interval. The WS-3 level was also up-regulated in salicylic acid treated samples but WS-1was enhanced more in comparison to methyl jasmonate (MeJA) treated samples. All values obtained were means of triplicate with standard errors. Values ofWS-1andWS-3were considered significant at *p<0.01 level and **p<0.01 levels.

Increasing trend of *Ws*DWF1 transcripts along with each development phase indicated possible role of *Ws*DWF1 in generating a reservoir of precursors for brassinolide and withanolide biosynthesis. Thus this study indicates *Ws*DWF1 as a possible responsive gene for signalling molecule(s) involved in withanolide biosynthesis in addition to its established role in brassinolide biosynthetic pathway.

2.11 Molecular cloning and functional characterization of *WsCYP710A* from *Withania somnifera* (L.) Dunal: An important gene of sterol biosynthetic pathway

Arti Sharma, Satiander Rana and Surrinder K. Lattoo

Cytochrome P450s form a huge superfamily of heme-containing monooxygenases present in all domains of life. These are pivotal in detoxification of xenobiotics, drug metabolism, assimilation of carbon sources and formation of secondary metabolites. Presently, there are more than 18500 P450 genes that have been identified across all the kingdoms of life. Reactions catalyzed by P450s can be broadly classified into hydroxylation epoxidation, oxidations, and reduction reactions which take place under conditions of limited oxygen assuming that an alternate electron acceptor is available. Elucidation of the physiological functions of many P450s in plants is still incomplete, but available evidence suggests their involvement in the synthesis of wide variety of secondary metabolites in plants. In an endeavour towards elucidation of role of various P450s in withanolide biosynthesis, present study entails cloning and characterization of *WsCYP710A* from *Withania somnifera*. Full length primers from start codon to stop codon resulted in amplification of 1506 bp ORFs encoding 501amino acid residues, respectively (Figure 2.11.1). The sequence information of cloned genes designated as *WsCYP710A* was submitted to NCBI GenBank Database (GenBank *Acc No.* KC008574). In addition to ORFs, full length cDNAs of *WsCYP710A* comprises of an upstream un-translated region (UTR; 430 bp) whereas downstream to stop codons of *WsCYP710A* contain 218 bp non-coding region having poly-A tail (Figure 2.11.2).

Figure 2.11.1: Nucleotide and deduced amino acid sequences. *WsCYP710A.* The first ATG is the start codon and stop codons are indicated by asterisk. The 5′-UTR and 3′-UTR regions are highlighted in green

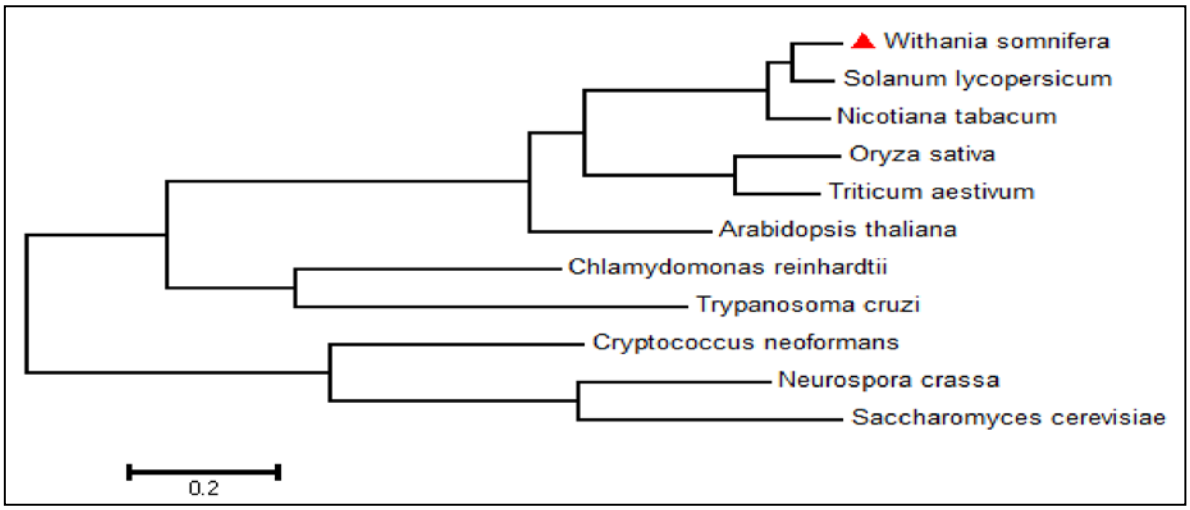


Figure 2.11.2 Phylogenetic analysis of deduced amino acid sequence of *Ws*CYP710A was inferred using the Neighbor-joining method using MEGA 5 software. A total of 11 sequences including *Withania somnifera* were used for analysis.

To ascertain the degree of evolutionary relatedness, Neighbour-joining phylogenetic tree was constructed with MEGA 6.0 software from the ClustalW alignment of *Ws*CYP710A with a number of homologous P450 sequences of different plants retrieved from the NCBI GenBank database (Figure 2.11.3). To study the *WsCYP710A* gene expression pattern and levels in different tissues of *W. Somnifera*, total RNA of leaves, stalks, roots, flowers and berries (unripen) from four month old plant was used as template for quantitative real-time PCR. The results showed that *WsCYP710A* expressed constitutively with varying expression levels in different tissues as depicted in Figure 3. *WsCYP710A* was found to be transcribing highest in leaves among all the tissues. The expression pattern of *WsCYP710A* is in agreement with the higher content of withanolides in leaves of *W. somnifera* as reported earlier and probably indicates involvement of *WsCYP710A* for driving the biosynthesis of withanolides.

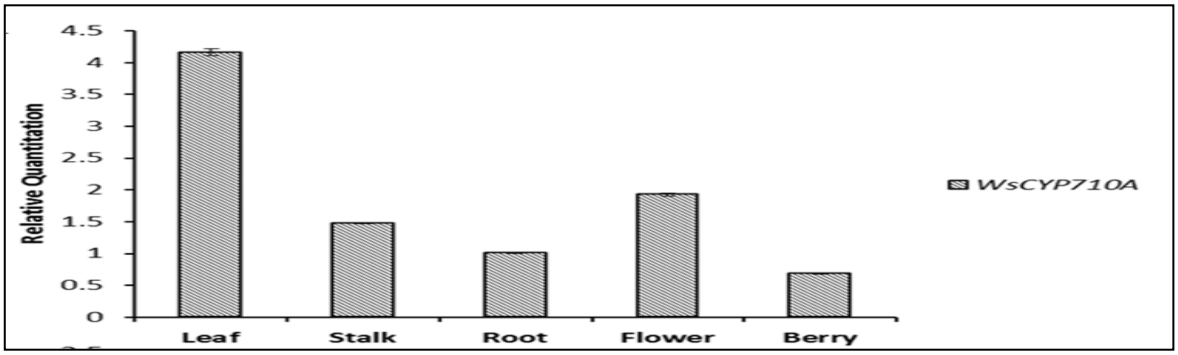


Figure 2.11.3 Quantitative assessment of the expression of *WsCYP710A* in different tissues of *Withania somnifera*. Data were compared and analysed with analysis of variance (*ANOVA*). Values are means, with standard errors indicated by bars, representing three independent biological samples, each with three technical replicates.

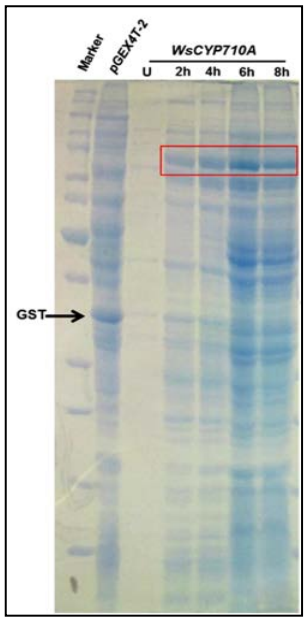


Figure 2.11.4 Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE: 10%) pattern of proteins obtained from *E.coli* BL21 (DE3) transformed with pGEX-*WsCYP710A*.

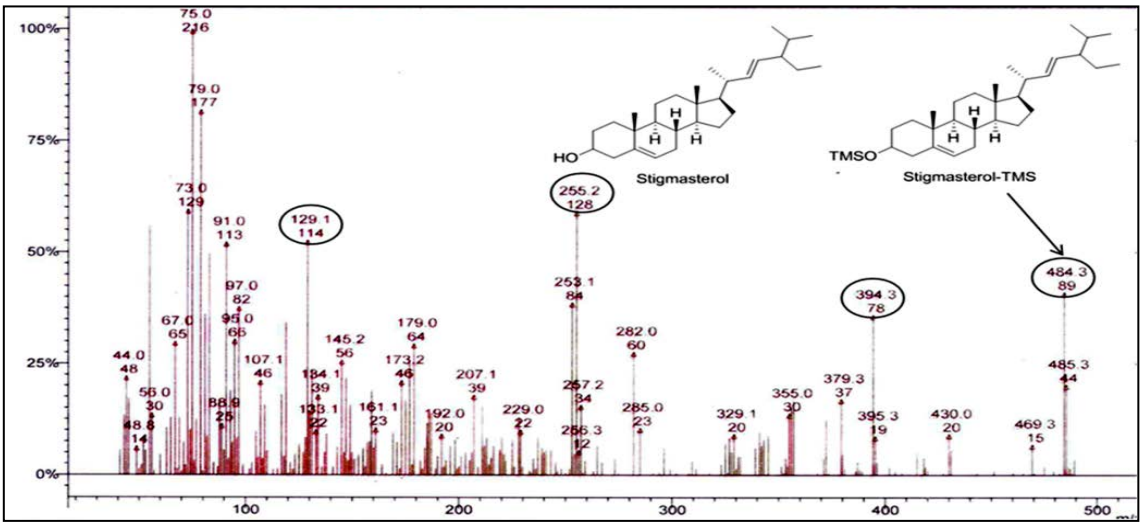


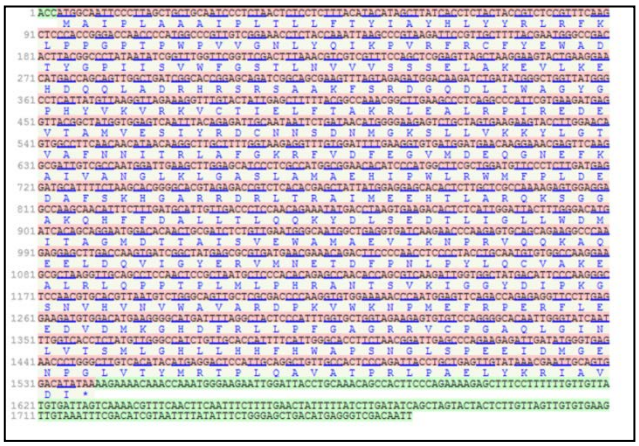
Figure 2.11.5: GC-MS total ion chromatogram of enzyme reaction from the microsomes expressing *Ws*CYP710A. The pattern of fragment ions (marked in circles) with m/z values of 484, 394, 255 and 129 were attributed to stigmasterol. Entire coding sequences of *WsCYP710A* cDNAs was expressed in *Escherichia coli* BL21 (DE3) using pGEX4T-2 expression vector. The recombinant expression vector with the inserted *WsCYP710A* in *E.coli was*purified in sufficient quantity for further characterization studies. Subsequently, the respective gene was cloned into pDS472a vector and transformed into *S. pombae*. For characterization, the microsomes containing *Ws*CYP710A were isolated and incubated with β-sitosterol. After 2 h of incubation, the product formed was dervatized with 1:1 mixture (40 µL) of pyridine and *N,O*-bis (trimethylsilyl) trifluoroacetamide containing 1% (v/v) trimethylchlorosilane at 90°C for 1 h to make it volatile. Finally, the derivatized product was fractionated three times with ethyl acetate and analysed using GC-MS. The pattern of fragment ions with m/z values of 484, 394, 255, and 129 were attributed to stigmasterol.

2.12 Molecular characterization of two A-type P450s, *WsCYP98A* and *WsCYP76A* from *Withania somnifera* (L.) Dunal: Expression analysis and withanolide accumulation in response to exogenous elicitations

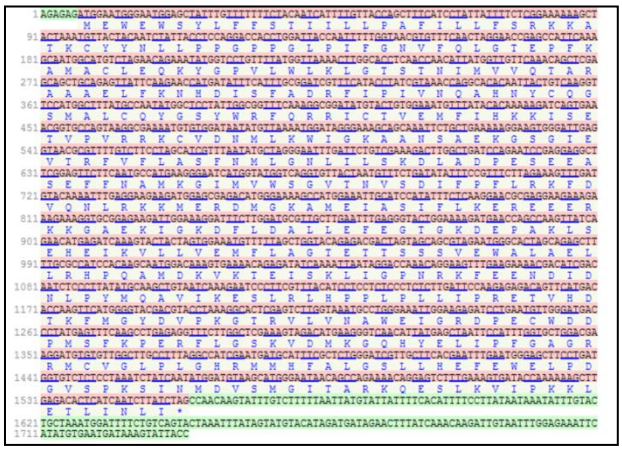
Satiander Rana, Niha Dhar and Surrinder K. Lattoo

Pharmacological investigations position withanolides as important bioactive molecules demanding their enhanced production. Therefore, one of the pivotal aims has been to gain knowledge about complete biosynthesis of withanolides in terms of enzymatic and regulatory genes of the pathway. However, the pathway remains elusive at the molecular level. P450s monooxygenases play a crucial role in secondary metabolism and predominantly help in functionalizing molecule core structures including withanolides. Due to diverse versatility of P450 in catalysing the regio and stereo-specific reactions, they are potential targets for industrial biocatalysis. P450s have been applied in industry for the investigation of new drugs, medicine or xenobiotics. Because of the remarkable variety of chemical reactions catalysed and enormous number of substrates attacked, P450s have earned the reputation of "the most versatile biological catalysts in nature". Identification and characterization of P450s is essential for the elucidation of various biosynthetic pathways.

Figure 2.12.1 **1A & 1B** Nucleotide and the deduced amino acid sequence of *WsCYP98A* (A) and *WsCYP76A* (B) from *Withania somnifera*. The start codon (ATG) present at 4th and 7th position whereas stop codons at 1552, 1537 bp, respectively.



1A



1B

In an endeavour towards identification and characterization of different P450s, we have cloned and characterized two A-type P450s, *WsCYP98A* and *WsCYP76A* from *Withania somnifera*. Full length cDNAs of reading frames of 1536 and 1545 bp encoding 511 (58.0 kDa) and 515 (58.7 kDa) amino acid residues, respectively (Fig. 1A &B). To ascertain the degree of evolutionary relatedness, Neighbour-joining phylogenetic tree was constructed with MEGA 6.0 software from the ClustalW alignment of *Ws*CYP98A and *Ws*CYP76A with a number of homologous P450 sequences of different plants retrieved from the NCBI GenBank database. *Ws*CYP98A and *Ws*CYP76A corresponded to two separate phylogenetic clans in accordance with the amino acid similarity among their proteins (Figure 2.12.2). Entire coding sequences of

WsCYP98A and *WsCYP76A* cDNAs were expressed in *Escherichia coli* BL21 (DE3) using pGEX4T-2 expression vector. The ORFs were released from pJET-*WsCYP98A* and pJET-*WsCYP76A* using *BamHI/Sal*I restriction enzymes, and inserted into vector pGEX4T-2.

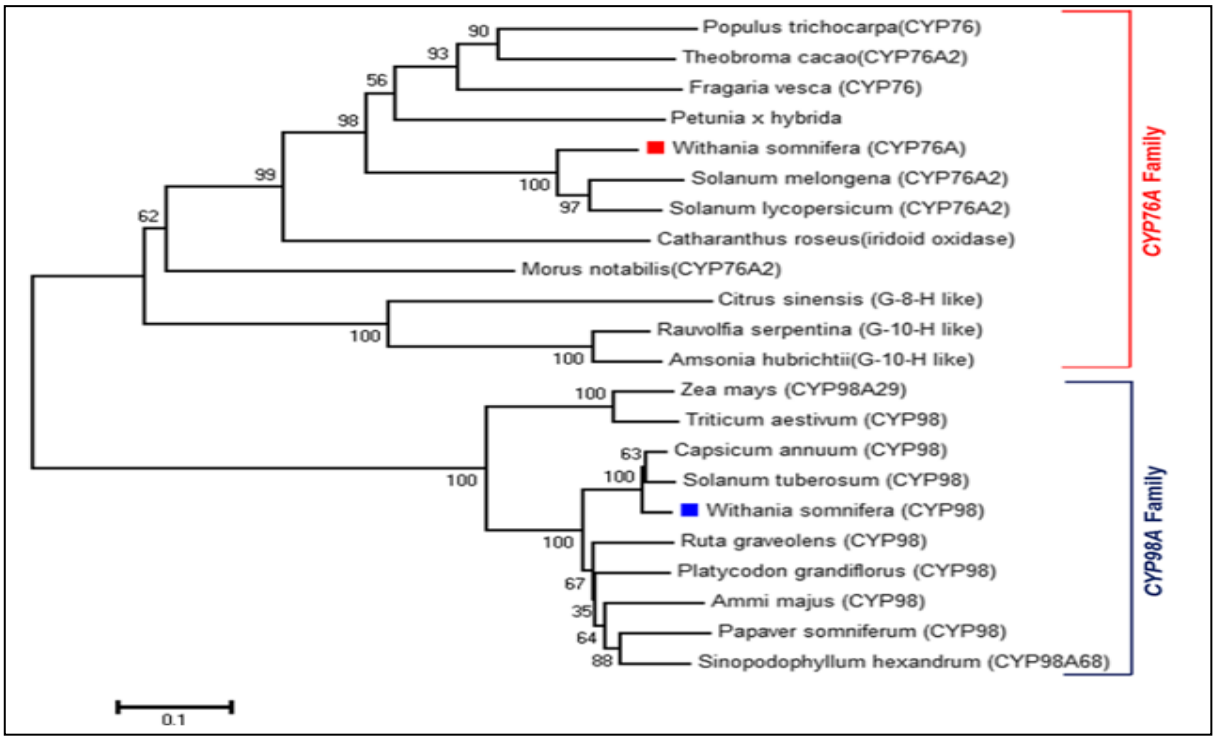


Figure 2.12.2 Phylogenetic analysis of deduced amino acid sequences of *WsCYP98A* and *WsCYP76A* was inferred using the Neighbour-joining method employing MEGA 6.0 software. For *WsCYP98A* total of 10 sequences and for *WsCYP76A*, 12 sequences including *Withania somnifera* were used for analysis.

WsCYP76A constructs were identified by PCR analysis and restriction digestion and *BamHI/Sal*I. Heterologous expression of proteins was induced with different concentrations of IPTG. SDS-PAGE analysis demonstrated that optimum expression of proteins was observed at 25 ˚C using 0.8 mM IPTG after 6-8 h of induction. The fusion protein having molecular weight of ~84.06 kDa and ~84.7 kDa appeared in the lysate of recombinant *E. coli* transformed with the expression cassettes pGEX-*WsCYP98A* and pGEX-*WsCYP76A*, respectively (Figure 2.12.3). To study *WsCYP98A* and *WsCYP76A* gene expression pattern in different tissues of *W. somnifera*, cDNA libraries were prepared separately from RNA samples extracted from leaves, stalks, roots, flowers and berries (unripen) of four month old plant. Tissue-specific cDNAs were used as templates for qRT-PCR. The results obtained showed both genes express widely in leaves, stalks, roots, flowers and berries with higher expression levels of *WsCYP98A* in stalks while *WsCYP76A* transcript levels were more obvious in roots (Figure 2.12.4).

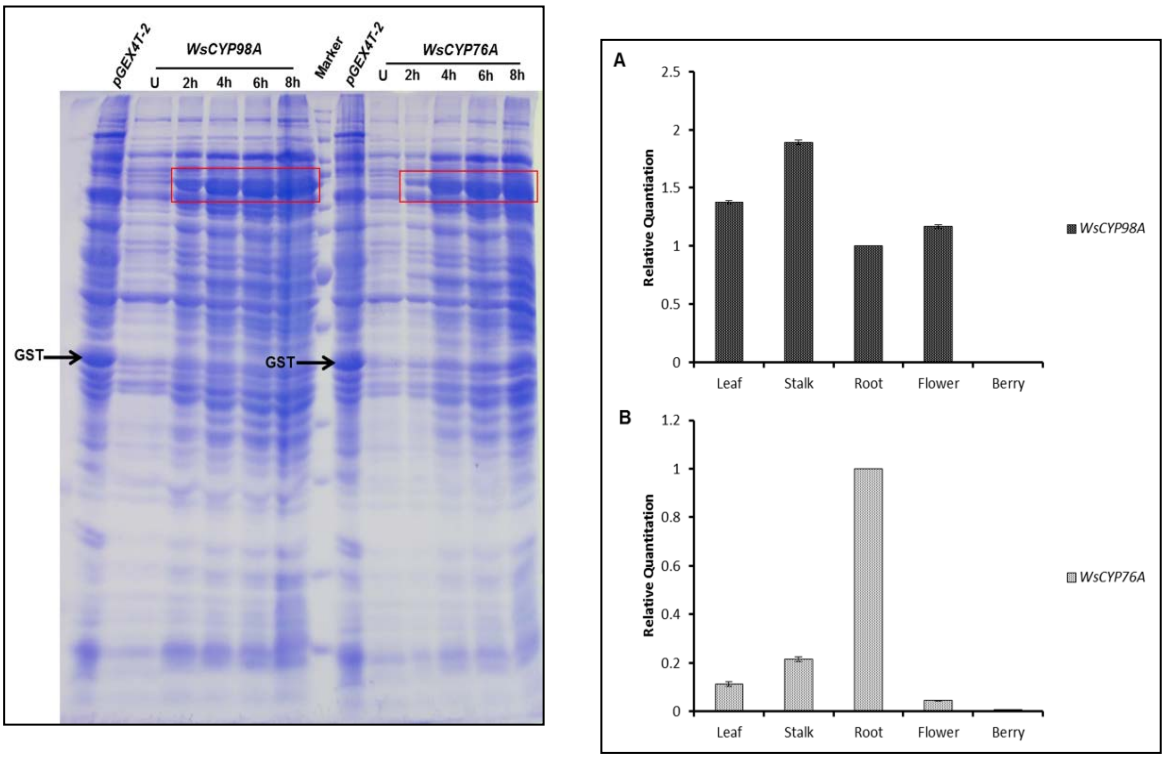


Figure 2.12.3 Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE: 10%) pattern of proteins obtained from *E.coli* BL21 (DE3) transformed with pGEX-WsCYP98A and pGEX-WsCYP76A.

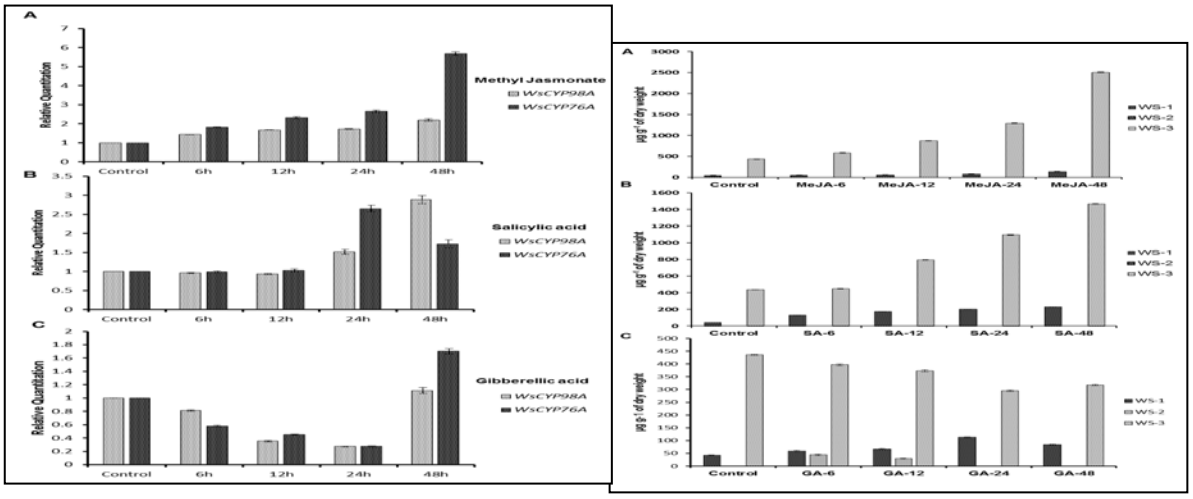


Figure 2.12.4 Quantitative assessment of the expression of (A) WsCYP98A and (B) WsCYP76A in different tissues of Withania somnifera. Data were compared and analysed with analysis of variance (ANOVA). Values are means, with standard errors indicated by bars, representing three independent biological samples, each with three technical replicates.

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2.13 Transcript and protein profiling of branch-point oxidosqualene cyclases in response to elicitation and diversion of precursor 2, 3-oxidosqualene towards withanolide biosynthesis

Niha Dhar, Satiander Rana, Aashiq Hussain, and Surrinder K. Lattoo

Withania somnifera (*Ws*) represents a rich repository of bioactive molecules in the form of withanolides which are being positioned as promising lead molecules for screening against various critical diseases and ailments. This prospect demands copious production of withanolides. As branch points present favourable gene targets for metabolic engineering of such imperative secondary metabolites. Therefore, three candidate oxidosqualene cyclases (OSC) genes namely cycloartenol synthase (*Ws*OSC/CS), β-amyrin synthase (*Ws*OSC/BS) and lupeol synthase (*Ws*OSC/LS) covering different branches of withanolide biogenesis were isolated and characterized. To have an insight into the regulatory mechanism of the three OSCs, our previous work involved isolation and analysis of various *cis*-acting elements in promoter regions of *Ws*OSCs. *Cis*-acting regulatory elements and their corresponding transcription factors constitute one of the transcriptional regulatory mechanisms induced by different environmental and extracellular conditions to help the plants in adaptive strategies. Therefore, to investigate how these putative *cis*-regulatory motifs regulate the three OSC expression, elicitations mediated by methyl jasmonate (MeJA) and gibberellic acid (GA_3) and microbe-derived exogenous elicitor yeast extract (YE) were performed. The regulation of cellular processes takes place at different levels including transcription, RNA processing, translation and post-translational modification. Consequently, examination of translational differences along with mRNA measurements is imperative for a better interpretation of obtained results. For studying the same, *in vitro* cultures of *Ws* were adopted to examine the variation in the accumulation of *Ws*OSC/BS, *Ws*OSC/LS, and *Ws*OSC/CS mRNA and protein on elicitor treatment of plant-derived endogenous elicitors methyl jasmonate (MeJA) and gibberellic acid (GA_3) and microbe-derived exogenous elicitor yeast extract (YE) using semi-quantitative method and western blot analysis. MeJA elicitation significantly increased the withaferin-A (WS-3) accumulation over a period of 48 h. Although the OSC mRNA expression model in case of GA_3 coincided with MeJA treatment, the total withanolide accumulation demonstrated a regular drop with increasing time-course. This may be attributed mainly to the decrease in *Ws*OSC/CS protein concentration as evident from the western blot study. Nevertheless, transcript abundance of *Ws*OSC/BS showed a rise which hinted towards the decrease in the total substrate availability for *Ws*OSC/CS but at protein level *Ws*OSC/BS expression declined with increasing time intervals. Thus possibly substantiating the drop in WS-3 concentration due to decreased *Ws*OSC/CS protein availability. Interestingly, microbe-derived exogenous YE elicitor played a role of negative regulator for the two competitive OSCs of *Ws*OSC/CS (*Ws*OSC/BS and *Ws*OSC/LS) at both the protein and mRNA levels, whereas *Ws*OSC/CS showed no change in its transcript or protein expression in response to YE. However, there was significant increase in withanolide concentration with YE in comparison with MeJA treatment. Plausibly, the down-

regulation of *Ws*OSC/BS and *Ws*OSC/LS leads to rearrangement of metabolic fluxes wherein bulk of 2, 3-oxidosqualene substrate pool shifts toward *Ws*OSC/CS, leading to much improved withanolide yields (Figure 2.13.1 and 2.13.2).

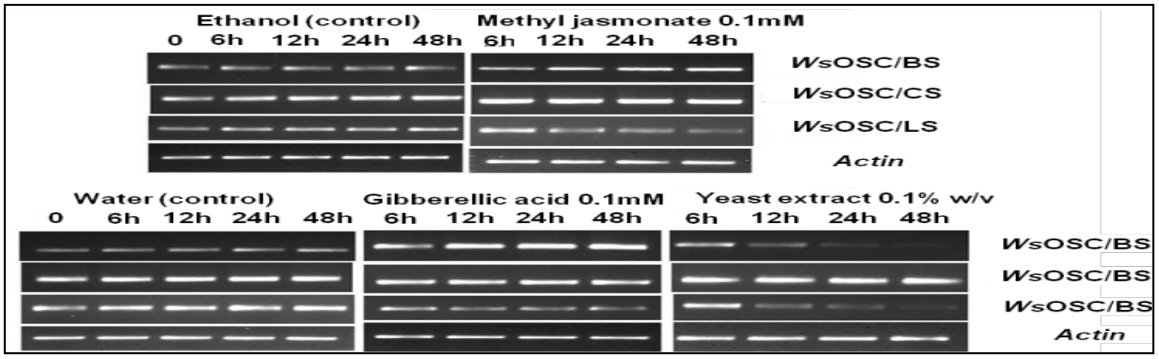


Figure 2.13.1. Transcript profiles of *Ws*OSCs in response to elicitor treatments. (A) Time courses of *Ws*OSC/BS, *Ws*OSC/LS and *Ws*OSC/CS expression in micropropagated *Withania somnifera* elicited by methyl jasmonate (MeJA; 0.1 mM), gibberellic acid (GA3; 0.1 mM) and yeast extract (YE; 0.1% w/v). β-actin was kept as endogenous control

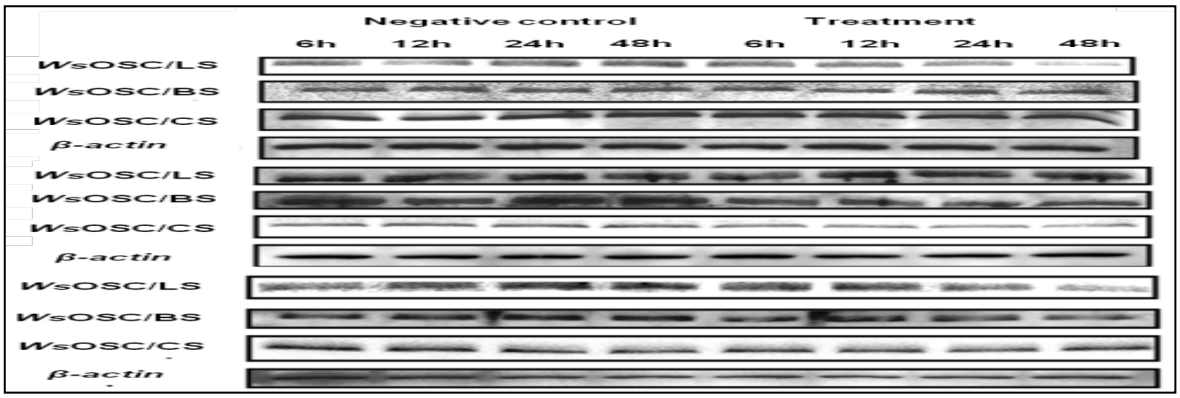


Figure 2.13.2. Western immunoblot of *Ws*OSCs in response to elicitor treatments. *A–C*, time courses of *Ws*OSC/BS, *Ws*OSC/LS, and *Ws*OSC/CS protein expression in micropropagated *W. somnifera* elicited by MeJA (*A*, 0.1mM), GA3 (*B*, 0.1mM), and YE (*C*, 0.1% w/v).

Induced culture of *Shizosaccharomyces pombe* was used for purifying the three recombinant WsOSC proteins. The OSCs expressed as GST tag fusion proteins in *S. pombe*, which enabled complete purification based on the principle of affinity chromatography using the glutathione-Sepharose beads. The purified fusion protein bands of *Ws*OSC/BS, *Ws*OSC/LS, and *Ws*OSC/CS were observed at ~113 kDa on SDS-PAGE, which coincided with the calculated molecular mass of the three proteins in addition to the 26-kDa GST tag (Figure 2.13.3). Furthermore, recombinant purified proteins were used for investigating the kinetic properties of *Ws*OSCs. Purified *Ws*OSC/BS, *Ws*OSC/LS, and *Ws*OSC/CS used 2, 3-oxidosqualene as substrate in independent reactions. As the substrate concentration was increased, the amount of β-amyrin, lupeol, and cycloartenol produced also increased. Vmax of each purified protein was also calculated. This was explained by Michaelis-Menten plots (Figure 2.13.4). The apparent Km value for *Ws*OSC/BS, *Ws*OSC/LS, and *Ws*OSC/CS was 38.48, 100.4, and 99.51 µM, respectively, thus showing that *Ws*OSC/BS has higher affinity toward 2,3-oxidosqualene followed by *Ws*OSC/CS and *Ws*OSC/LS. Among the three OSCs, *Ws*OSC/BS was observed to possess a higher specific activity of 2.9 µM/min/ml as compared with 2 and 1.43 µM/min/ml of *Ws*OSC/LS and *Ws*OSC/CS correspondingly.

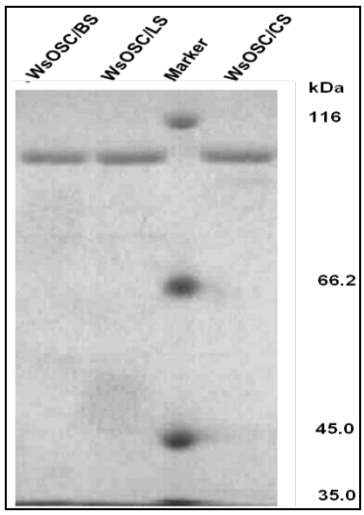


Figure 2.13.3: SDS-PAGE profile of purified recombinant proteins. *First lane*, purified recombinant GST-fused *Ws*OSC/BS; *second lane*, purified recombinant GST fused *Ws*OSC/LS; *third lane*, standard protein marker; *fourth lane*, purified recombinant GST-fused *Ws*OSC/CS.

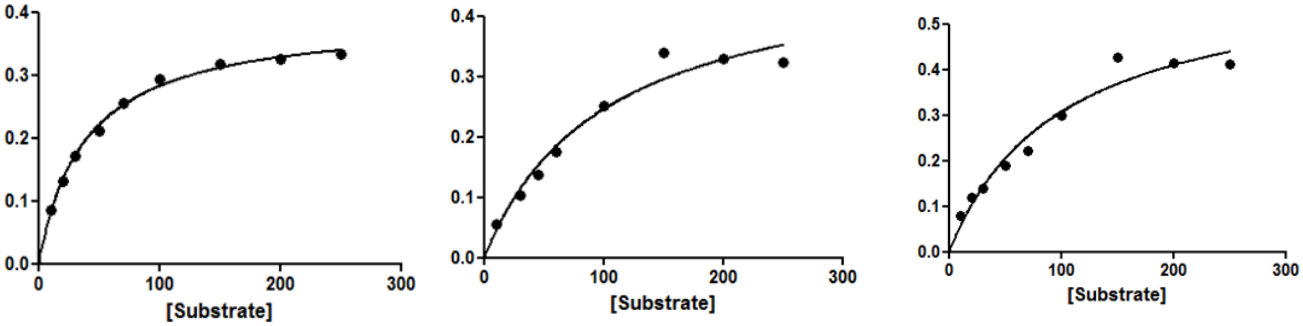


Figure 2.13.4: Kinetic study of *Ws*OSC/BS, *Ws*OSC/LS, and *Ws*OSC/CS. *A–C*, Michaelis-Menten plots of *Ws*OSC/BS (A), *Ws*OSC/LS (B) and *Ws*OSC/CS (C) with 2,3-oxidosqualene. Kinetic parameters *Km* and *V*max were obtained by fitting the data in the Michaelis-Menten equation by nonlinear regression analysis using GraphPad Prism 5 software.

Copy number validation of three OSC genes in the Ws genome was performed using genomic southern analyses with DIG-labeled full-length probes for *Ws*OSC/BS, *Ws*OSC/LS, and *Ws*OSC/CS. Genomic DNA was digested using non-cutter and single-cutter restriction enzymes, subjected to electrophoresis, and transferred to a positively charged membrane for hybridization with probes. For *Ws*OSC/BS and *Ws*OSC/LS, single bands were scored for DNA digested with SpeI and SalI enzymes and two bands with ScaI and EcoRI and NcoI and EcoRV, respectively (Figure 12.13.5, A and B). The results obtained suggest that Withania genome possibly contains a single allele for both *Ws*OSC/BS and *Ws*OSC/LS. In *Ws*OSC/CS, two bands were detected in XbaI and XhoI-digested DNA, and more than two were detected with HindIII and DraII digestion (Figure 12.13.5C). The Southern blot results suggest that an additional gene copy of WsOSC/CS may exist in the Ws genome. In view of the fact that cycloartenol synthase participates in both sterol and withanolide biosynthesis, the results suggest that cycloartenol synthase confronts the high metabolic demand in terms of gene copy number. Possibly, dual copies of *Ws*OSC/CS might be involved in carrying primary and secondary functions separately in *Ws*.

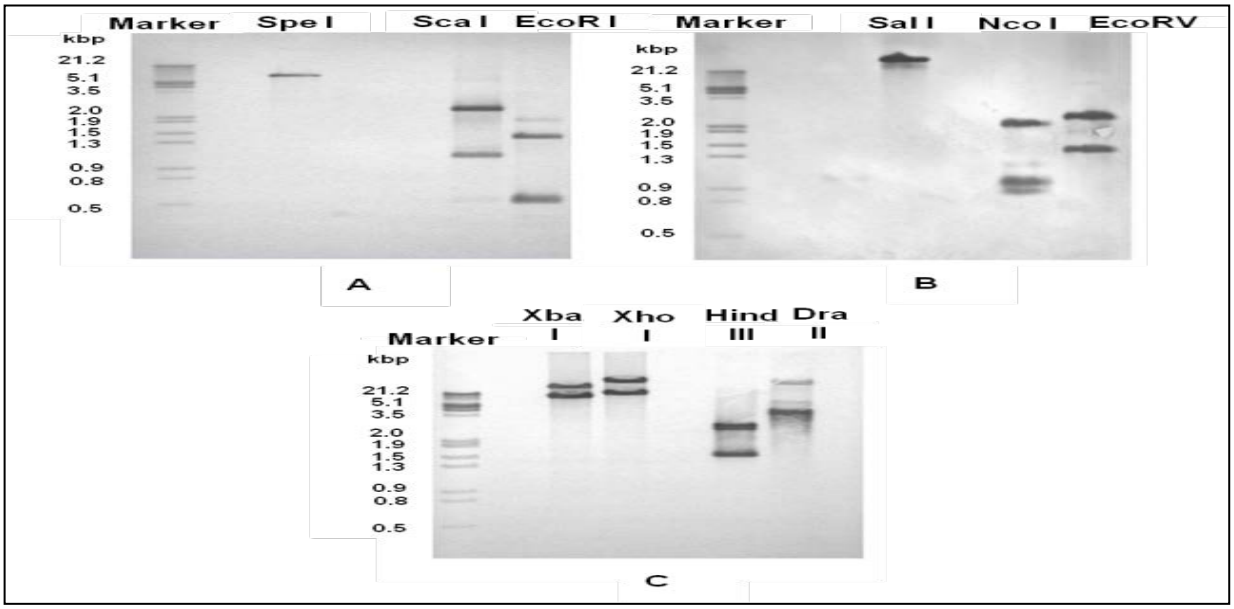


Figure 2.13.5 Southern blot analysis of *Ws*OSC/BS (*A*), *Ws*OSC/LS (*B*), and *Ws*OSC/CS (*C*). *W. somnifera* genomic DNA was digested with SpeI (non-cutter) and ScaI and EcoRI (single-cutter) for *Ws*OSC/BS, with SalI (non-cutter) and NcoI and EcoRV (single-cutter) for *Ws*OSC/LS, and with XbaI and XhoI (non-cutter) and HindIII and DraII (single cutter) for *Ws*OSC/CS; separated on 0.8% agarose gel; blotted onto a nylon membrane; and hybridized with DIG-labeled ORF of *Ws*OSC/BS, *Ws*OSC/LS, and *Ws*OSC/CS as probes.

2,14 Terpenoid and flavonoid spectrum of in vitro cultures of Glycyrrhizaglabrarevealed high chemical heterogeneity:platform to understand biosynthesis.

Saima Khan, Pankaj Pandotra, Malik MuzafarManzoor, ManojKushwaha, Rajni Sharma, Shreyansh Jain, Ashok Ahuja, Vishal Amancha, SashiBhushan, Santosh Kumar Guru, Ajai Prakash Gupta , Ram Vishwakarma, Suphla Gupta.

Simultaneous qualitative and quantitative assessment of eight flavonoids and two terpenoids were performed in fourteen in vitro raised morphogenic cultures of Glycyrrhizaglabra. Our study revealed that the spectrum and production of ten compounds, under investigation, were higher in organized tissue than the undifferentiated mass, however, aerial portions of the in vitro raised plants (leaf and stem) were found to be devoid of therapeutically relevant triterpenoid, glycyrrhizin. A correlation was observed between cell maturation, morphological ifferentiation and glycyrrhizin accumulation. Mature stolons (4 months) were characterizedby the maximum accumulation of glycyrrhizin (8.60 lg/mg) in in vitro plantlets. The cytotoxic effect of the extracts evaluated against a panel of human cancer cell lines (in vitro) indicated that the pancreatic cell line (MIAPaCa-2) were sensitive to all the fourteen extracts investigated. To the best of our knowledge this is the first comprehensive report relating plant growth regulators to metabolite spectrum and cytotoxic assessment in in vitro raised G. glabracultures. Overall, our findings demonstrated that the metabolite spectrum of in vitro raised morphogenetic lines, under different stages of maturation, might offer a platform to understand the regulatory aspects of the concerned metabolite pathway and their consequent role in differentiation.

In vitro chemical spectrum:

The biosynthetic potential of plantlets and morphogenetically distinct culture lines were analyzed for the two triterpenoids and eight flavonoids in different stages of maturation and at various time intervals.The metabolite spectrum of triterpenoids and flavonoids in the aerial and underground parts of plantlets were found to deviate significantly. The biologically active triterpenoid saponins (glycyrrhizin and 18-a glycyrrhetinic acid) were not detected in the aerial part of the 4 months old in vitro raised plantlet (TC1). However, underground rhizome showed presence of (8.60 lg/mg) glycyrrhizin. Among the organ culture analyzed, roots originating from in vitro plantlets, axenic root cultures (TR1 & TR2), yellow stolons (TC3), brown stolons (TC4) and brown root callus (TR2 & H7), produced glycyrrhizin under in vitro conditions. Basal medium and plant growth regulators (PGRs) play a significant role in tissue morphogenesis, differentiation and secondary metabolite production. As most of the terpenoids and flavonoids were found in the licoriceroots, the role of basal media composition and PGR effecton root tissue differentiation and secondary metaboliteproduction was investigated in in vitro raised cultures of G.glabra. Five basal media namely, full strength Murashigeand Skoog (FMS), half strength MS (HMS), R medium(MS with different vitamin composition), White's (W) andGamborg's (B) media supplemented with seven (1–7) PGRcombinations were used to assess the response of in vitroraised root explants. Simultaneous quantification of all thelines in various combinations demonstrated that irrespectiveof the basal medium, the root explants evoked threetypes of morphogenetic response, in 47 % of the cultures(16/35). Five out of sixteen cultures showed a very weakresponse, either due to the browning of the callus or veryslow growth and hence were not studied further. Amongthe cultures responded, root +callus mixed morphologywas observed in 8 cultures (50 %) while pure callus andpure root morphology were seen in 5 and 3 PGR combinations,respectively.

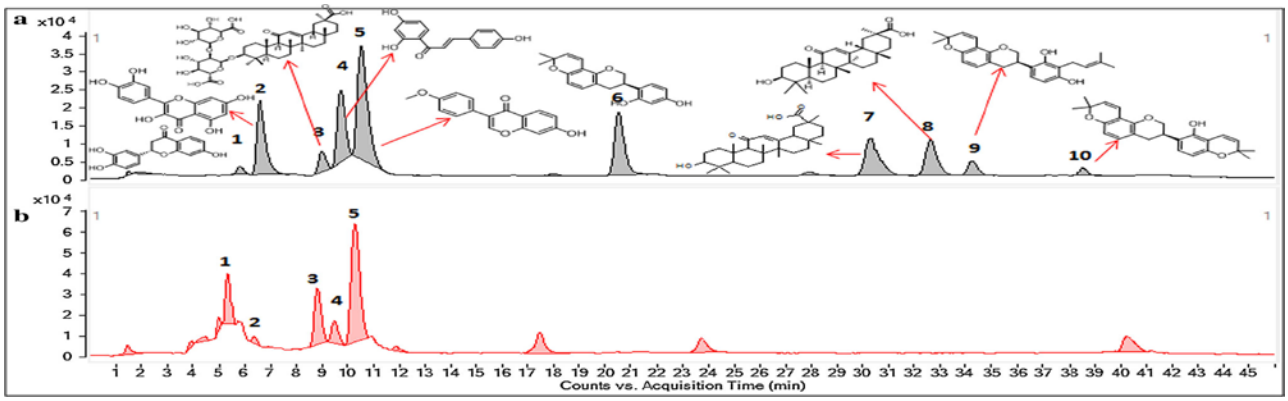
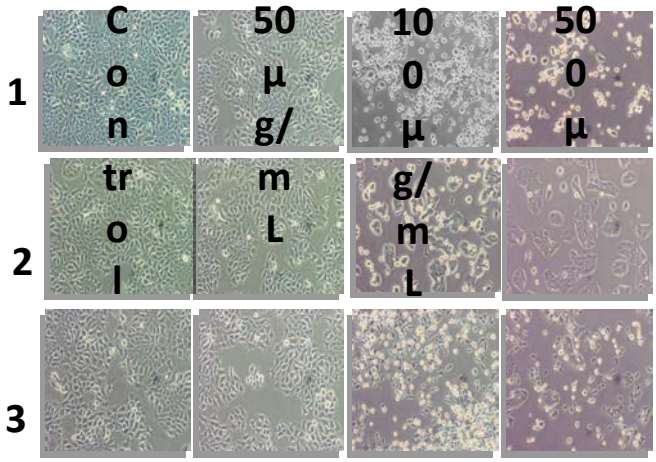


Figure. 2.14.1 Effect of basal media and growth regulators on morphogenesis

In vitro cytotoxic assay:

Flavonoids and terpenoids have been known to possess anticancer properties (Fukai et al. 2000; Zhang et al. 2009). Preliminary in vitro cytotoxicity of all the tested compounds (1–10) was evaluated by performing a comprehensive screening at a single dose of 100 lg/ml, employing MTT assay, against various human cancer cell lines encompassing human breast cancer (MCF-7), pancreatic cancer cell lines (MIA PaCa-2), lung cancer cell line (A549) colon cancer cell line (HCT-116). The results are summarized in Table 2 and expressed as a percentage (%) of growth inhibition. The results demonstrated that extracts induced cell growth inhibition ranged between nil to 44 % in breast cancer, 43 % in lung cancer and 11 % in colon cancer cell lines. Pancreatic cancer cell lines showed inhibition ranging between 2–19 %.



1-Aerial part; 2-Root; 3-Aerial + Root

3.0 DISCOVERY INFORMATICS

3.1. Ec GlmU fragment based studies

Rukmankesh, Amit Nargotra, Rashmi Sharma, Inshad Ali Khan

In continuation to our earlier work on the target GlmU from E coli and Mtb, further work was carried out with respect to the in silico structural modification, similarity analysis and similarity search of the best actives.

Virtual modification based on fragment based studies for E coli GlmU Based on the binding site analysis, a total of five sites were recognized that were found to be reasonable for substitutions as suggested by the arrows (numbered as 1 to 5) in figure 3.1.1.

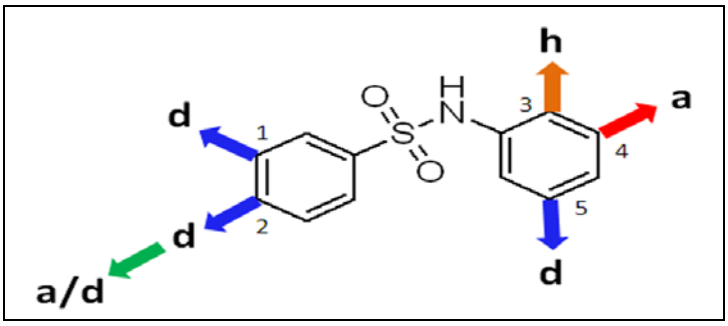
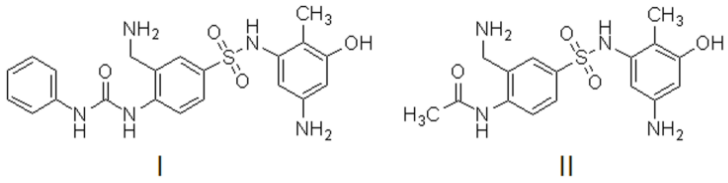
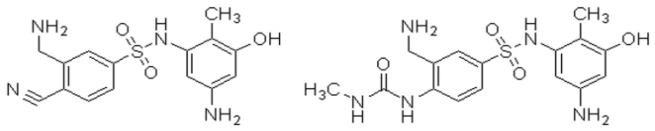


Figure 3.1.1. Sites for modification on the identified E coli GlmU inhibitory leads

At site 2, a major addition of groups was possible because of the presence of large vacant space near this site. At this site, H-bond donor groups were added followed by the addition of H-bond donor or H-bond acceptor groups, as suggested by the SiteMap surfaces. The resulting compounds from these substitutions were prepared using LigPrep. Docking of these compounds was performed so as to analyze the binding pose and shape complementarily with the target. The binding pose of the best hit (compound I) was analyzed w.r.t. the binding site surfaces. It was observed that, as suggested, the NH2 group of the added H-bond donor group –CH2-NH2 at site 1 was occupying the donor region of the SiteMap. Further the addition of bulkier group N-phenylurea (-NH-CO-NH-Ph) at site 2 caused the added groups to lie in the respective favorable regions. Similarly the added NH2 group at site 5 is occupying H-bond donor region. These all additions helped in increasing the interactions and the shape complementarily of the inhibitors with respect to the acetyltransferase site. Based on similar analysis 4 new compounds were proposed as the predicted inhibitors of the Ec GlmU acetyltrans ferase site as shown in figure 3.1.2.



I II

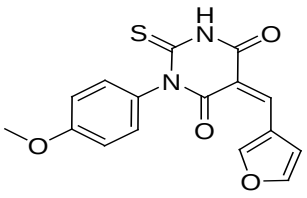


III IV

Figure 3.1.2. Identification of four potent inhibitor of E coli GlmU after lead optimisation studies

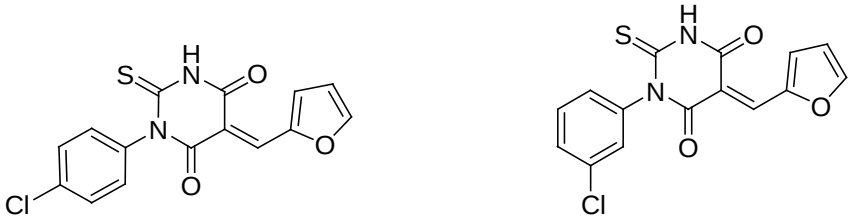
Similarity search analysis of the hits from Mtb GlmU

Based on the in vitro screening earlier, 93 compounds were found to have more than 20% inhibition of the acetyltransferase activity of Mtb GlmU at 100 µM. Out of these, 15 compounds showed more than 40% inhibition. Similarity analysis of all these compounds with respect to the training dataset (PubChem compounds) was also carried out in order to find the structural novelty of the identified hits. It was observed that 69 compounds out of 93 were having similarity value less than 0.5 with the training set compounds, whereas only 2 compounds had a maximum similarity of more than 0.8. Thus, this filtering methodology could help in the identification of 15 novel inhibitory leads of this target, directly from the repository. Compound ID 5810599 with 82.5% inhibition was the most active compound found in this assay (figure 3.1.3). Further, the dose-response assessment of this compound was carried out and the IC50 of this compound was found to be 9.018 ± 0.04 µM. Besides this, similarity analysis of all the 93 compounds was also carried out with respect to the activity range of the training set data. In total, 17 compounds were found to have similarity values >0.6 with the PubChem compounds. It is inevitable to miss out some active compounds (false-negatives), while screening any compound library using computational methods. One way to reduce the number of false-negatives is to revisit the compound library and identify the compounds similar to the most active in vitro hit. In order to fish out the possible actives that might have been missed during the in silico screening, the compounds similar to the most active identified inhibitory lead (5810599) were retrieved from the 20,000 compound library. The similarity search in this case was performed using a high Tanimoto coefficient of 0.7. Using this approach, 23 in silico hits were identified that were not screened in vitro earlier. These 23 hits were also evaluated using DTNB bioassay, which helped in the identification of 8 additional inhibitory leads with more than 40% inhibition. Out of these, 2 compounds viz. 5680705 and 5673625 (figure 4) exhibited the IC50 values of 27.65 ± 0.021 and 28.49 ± 0.027 µM respectively. It was found that both these compounds were highly similar to the query compound 5810599, except that a chlorobenzene group was attached to the thioxopyrimidine-dione instead of anisole.



5810599

Figure 3.1.3. Most potent E coli GlmU inhibitor identified after in vitro screening



5680705 5673625

Figure 3.1.4. Compounds possesing reasonable IC_{50} values after identified through similarity search of the most active in vitro hit.

3.2 Lead Optimization studies on EGFR

Priya Mahajan, Amit Nargotra, Nitasha Suri, Shashank K Singh

In continuation to our ealier work on EGFR lead optimization studies, docked poses of 87 common insilico identified hits were analysed on the basis of their binding pose with the target. The orientations of the hits were analyzed within the binding pocket, and twelve common structural moieties were identified on the basis of structural superimposition. These twelve structural moieties belong to imidazopyridine-carbohydrazide, indandione, thieno-pyrimidine, imidazol-pyridine, triazolopyrimidine amine, pyrazolo-quinolinone, purine, benzofuropyrimidine amine, purinone, coumarine and quinoxalinone derivative series. These structural moieties then served as templates for analysing the scope of modifications around them and for optimizing anti-EGFR activity. The unoccupied hydrophobic, H-bond donor and acceptor regions were analyzed within the binding pocket and the positions were identified for modifications on these structural moieties as shown in figure 3.2.1.

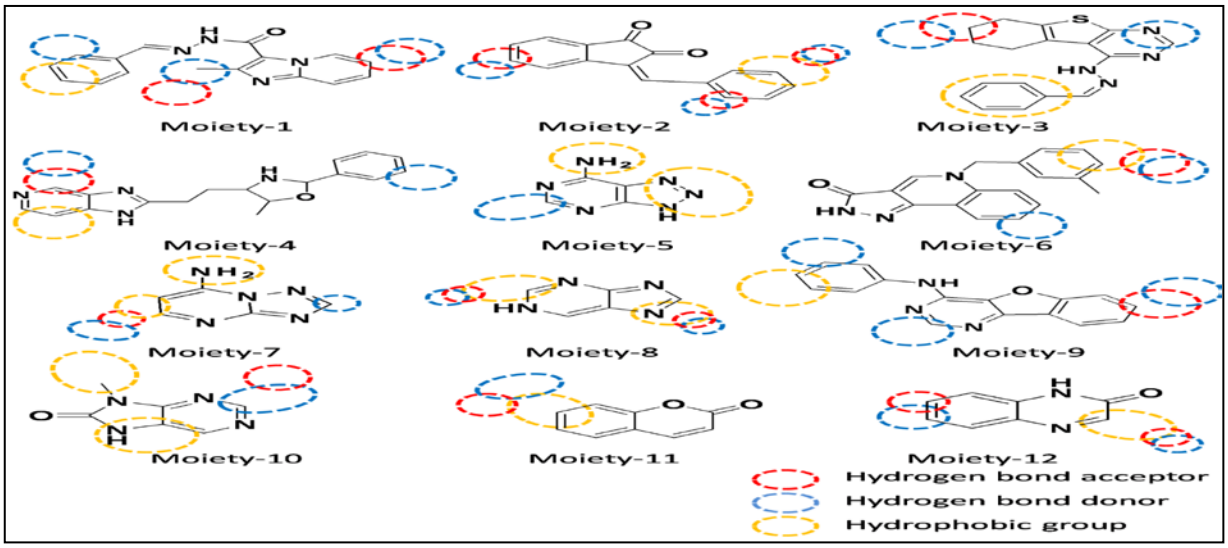


Figure 3.2.1. Scope of modification around the identified structural moieties.The yellow circle indicates the scope of adding hydrophobic groups, blue circle representsthescope of adding H-bond donor groups and red circle shows the scope of adding H-bond acceptor groups. Moiety-1and 2 are novel, moiety 3 to12 are reported for kinase activity whereas moiety-5 to 12 are already reported for anti-EGFR activity.

The above identified 12 structural moieties were selected for sub-structural database search using Scifinder (https://scifinder.cas.org/scifinder) to obtain the anti-kinase activity data for EGFR target. On analyzing the data retrieved from database search, first two structural moieties given in figure 5 are found to be novel for anti-kinase activity, moieties 3-12 were reported for inhibition of kinase activity, whereas moieties 5-12 are already reported for EGFR inhibition. Out of the eight reported scaffolds/structural moieties (moieties 5-12) for EGFR inhibition, four are in nano molar (nM) range. This also provides strength to the predictive competence of the in silico filters developed in the present study to identify novel and potential leads (inhibitors) from the drug like library for anti-EGFR activity.

3.3 Molecular modelling studies on PDE5

Priya Mahajan, Amit Nargotra, Sajad Hussain

An in silico driven screening programme was carried out on PDE5 which is a very well know therapeutic target for erectile dysfunction (ED) and hypertension. Notably, phosphodiesterase type 5 (PDE5), a hydrolase enzyme widely expressed in lung, smooth muscle, platelets and kidney, is responsible for the breakdown of cGMP through the hydrolyzation of cGMP to inactive metabolite 5'cGMP, resulting in the decrease of cGMP level. The diseases such as male erectile dysfunction (MED, the most commonly encountered form of sexual dysfunction in men), pulmonary hypertension, memory retention and diabetes are due to the lowering of cGMP level induced by PDE5. Therefore, development of PDE5 inhibitors recognized as a possible approach to treat such diseases. Here, we designed certain filtering criteria such as Filter 1(Pharmacophore models, e-Pharmacophore models, docking, substructure and similarity search of co-crystallized and PDE5known inhibitors) and Filter 2 (interaction of the top 100 hits with the PDE5 key residue Gln817) to screen the in-house library. The Structure Based Approach and Ligand Based Approach were applied for virtually screening the library, and the method is summarized in figure 3.3.1

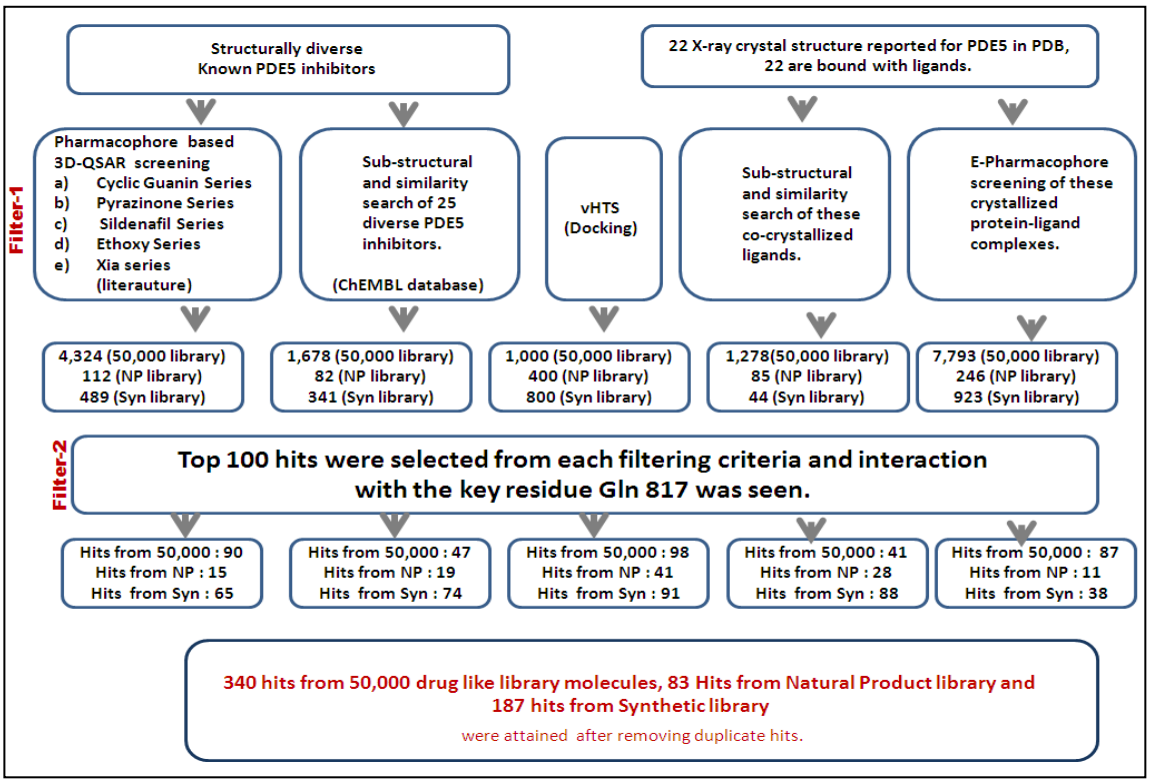


Figure 3.3.1 Screening methodology for identification of potent PDE5 inhibitors from the Institutional compound library.

Finally, the hits attained from in-house NP and Synthetic library were biologically validated and four compounds were found to be active for PDE5 inhibitors as shown in figure 3.3.2.

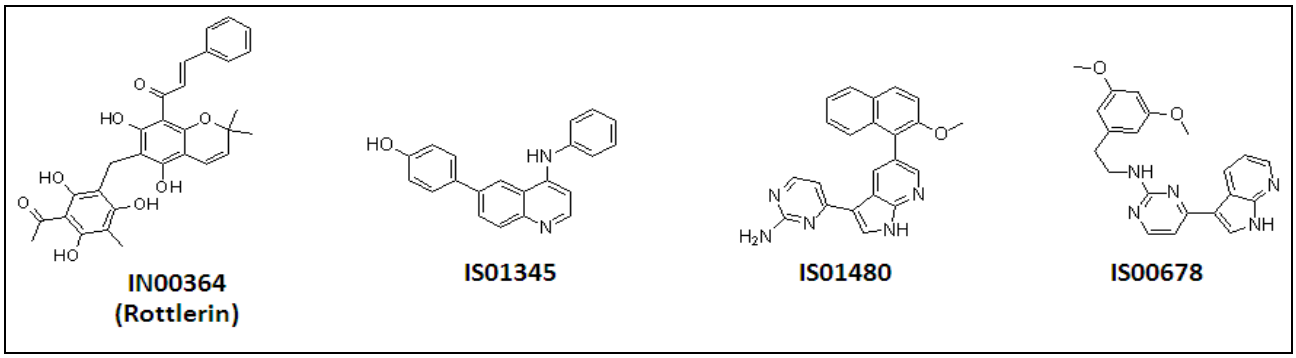


Figure 3.3.2 Compounds found active against PDE5 after in vitro screening

3.4 Updation of Stem cell database (MedchemDB)

Rakhi Talwar, Monika Gupta, Amit Nargotra, Ram Vishwakarma.

In continuation to our earlier efforts towards the development of MedchemDB, which is a systematic compilation of various pathways, crystal structures and target details related to the stem cell research, further activities have been carried out for improving the database. The portal is now enriched with updated information. Scaffolding of the identified inhibitors was also carried out and the same has been uploaded on the portal. The portal is now accessible over Internet at http://medchemdb.iiim.res.in /

Efforts are being carried out for obtaining the copyright for the MedchemDB portal.

3.5 Repository database updation and compound flow management

Monika Gupta, Amit Kumar, Amit Nargotra, Naresh Satti, Ram Vishwakarma

During the reporting period 35 Natural Products and 416 new chemical entities from the med chem projects have been added to the repository along with the HPLC/HPTLC profile. All these compounds are also incorporated into the database for sub-structural search. The outcome of this compound repository in various Institutional discovery activities is highlighted in figure 3.5.1

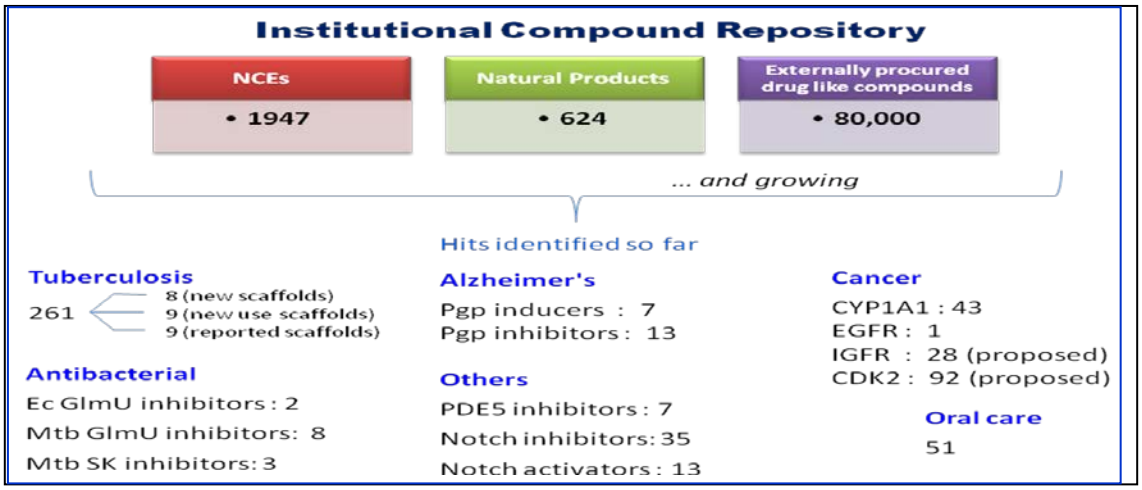


Figure 3.5.1. Discovery outcome of the Institutional compound repository

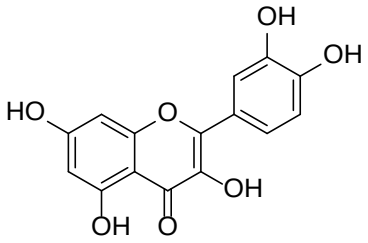
Random re-validation of the submitted HPLC data of the compounds in the repository was carried out by selecting every fifth compound from the library. In total, about 500 compounds of the library were selected for re-validation and the data for the same is also incorporated into the database. With regard to the biological evaluation, during the reporting period (January to November 2015), a total of 6034 compounds were issued for biological evaluation through this repository. Out of these, 5389 compounds were sent outside IIIM for evaluation against several established target assays. Further, the cytotoxic studies of the hits thus identified, is under process. 30000 more compounds have been ordered after diverse selection and in silico studies. This contains 15000 diverse set and 15000 targeted libraries for targets viz. i) GSK-3β, ii) Smo, iii) Gli, iv) EGFR, v) γ-Secretase, vi) AKT, vii) mTOR, viii) Tankyrase, ix) β-Catenin, x) FAK and xi) Pi3K.

4.0 NATURAL PRODUCT CHEMISTRY

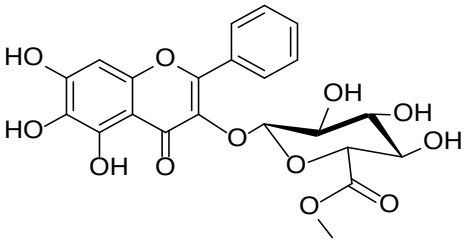
4.1 Extraction and isolation of chemical constituents from *Colebrookea oppositifolia*

Neha Sharma, N.K.Satti, Prabhu Dutt, M.K.Verma

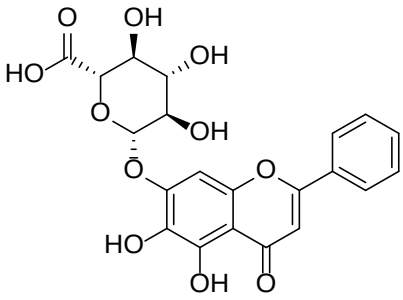
The chemical investigation of the leaves extract of the plant has resulted in the isolation of Quercetin; 5,6,7-Trihydroxy flavone -3-O-Glucuronide methyl ester (CO-2) and baicalin in addition to 8 compounds (Acteoside ; 5,7,4'-Trihydroxy flavone -3-O-Glucuronide (CO-1); 5,6,7-Trimethoxy flavone (CO-3); 5,6,7,4'-Tetramethoxy flavone (CO-4); β-Sitosterol glucoside; 5-hydroxy, 6,7,8-Trimethoxy flavone; 5-hydroxy- 6,7,8,4'-Tetramethoxy flavone; Hentriacontane) already isolated by column chromatography. Structures of the compounds have been established by spectral analysis.



Quercetin

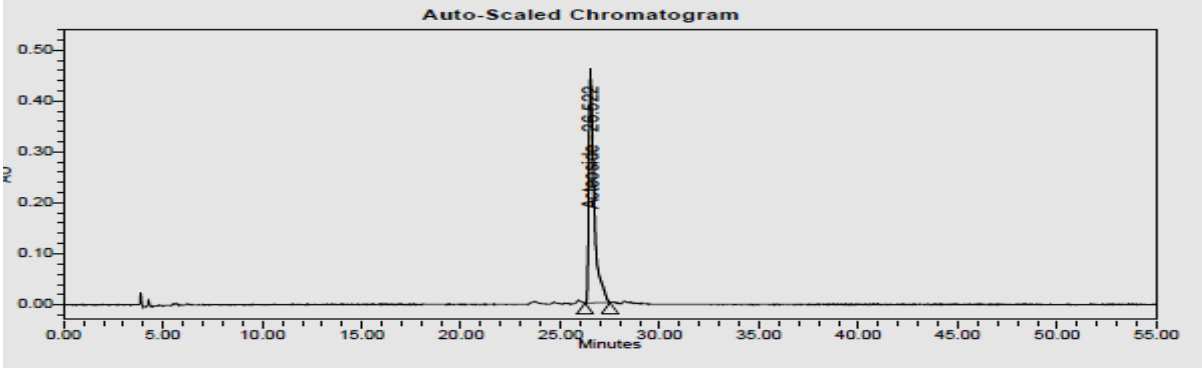


5,6,7-Trihydroxy flavone -3-O-Glucuronide methyl ester (CO-2)

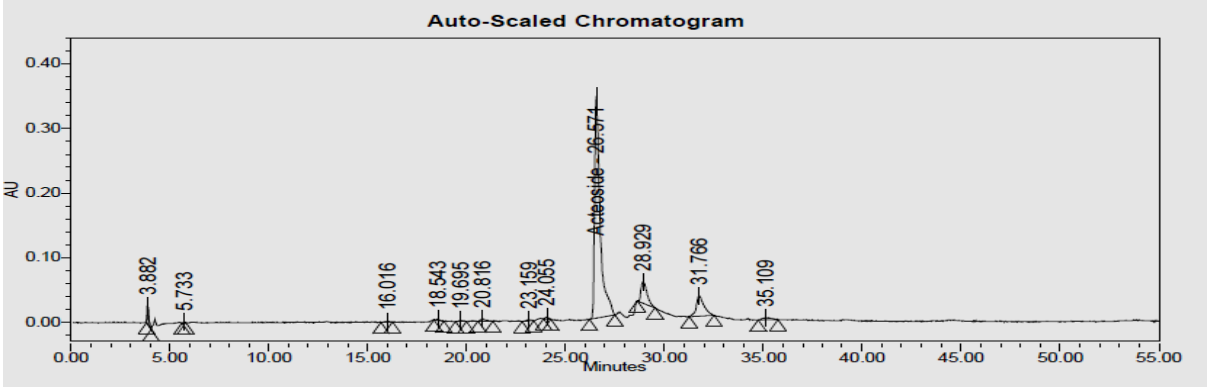


Baicalin

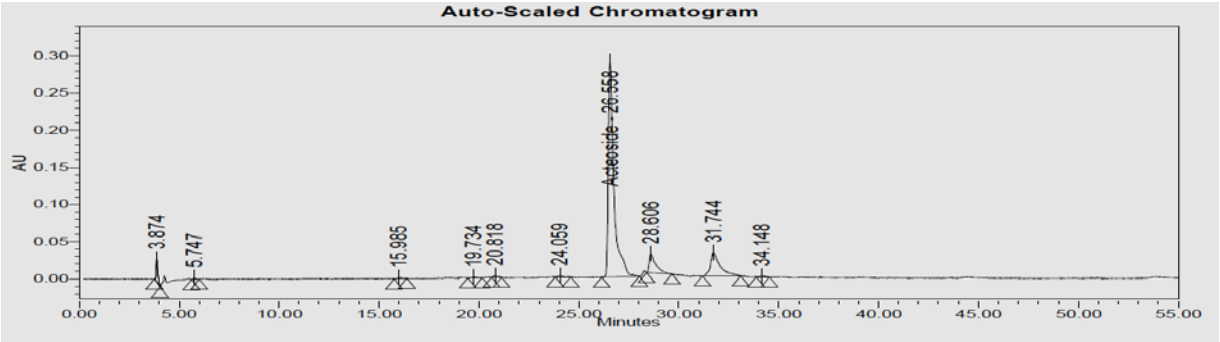
Comparison of acteoside content in wild and domesticated plant material of *Colebrookea oppositifolia* leaves in ethanolic extract by HPLC analysis



HPLC chromatogram of pure compound acteoside

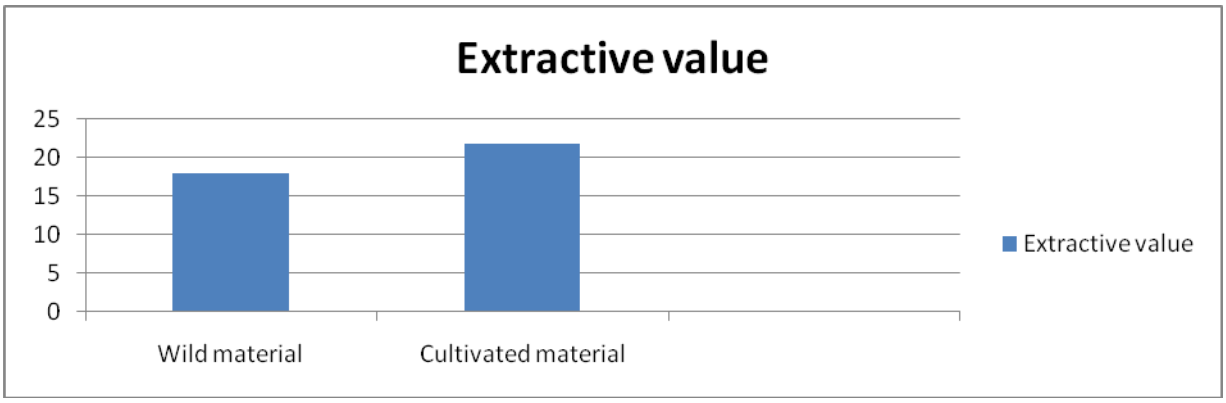


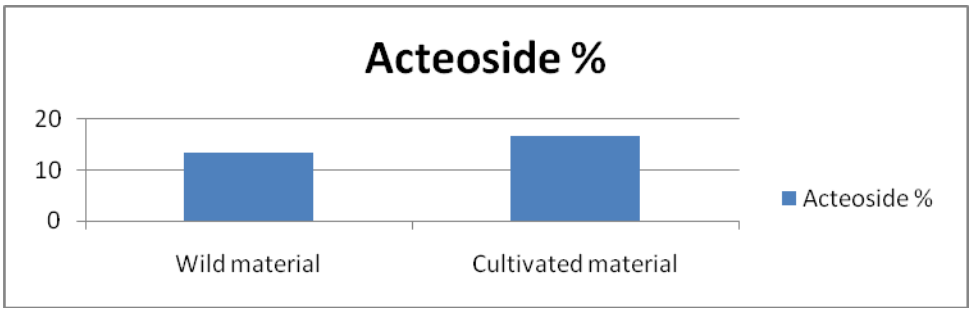
Cultivated material (HPLC chromatogram of ethanolic extract of *Colebrookea oppositifolia* leaves procured from IIIM Chatha Farm)



Wild material (HPLC chromatogram of ethanolic extract of *Colebrookea oppositifolia* leaves procured from Purmandal)

Sample	Extractive value %	Acteoside % in extract 5µl inj	10µl inj
RJM0862 (Chatha Farm)	21.8	16.781	16.853
RJM0862 (Purmandal) wild collection	18.0	13.343	13.791



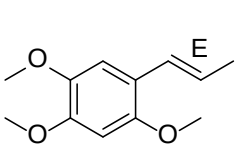


Conclusions: It has been observed from the aforesaid data that in the cultivated sample, there is an enhancement of extractive value about 3.8 % and an enhancement of acteoside content in the extract about 3.4 % as compared with wild sample.

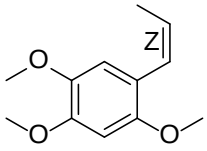
4.2 Extraction and isolation of chemical constituents from *Acorus calamus*

Chetan Kumar, N.K.Satti, Prabhu Dutt, M.K.Verma

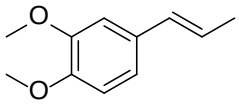
The chemical investigation of rhizomes extract of the plant has resulted in the isolation of α-asarone, β-asarone, Methyl isoeugenol, 2,4,5-Trimethoxy benzaldehyde , β-sitosterol, α-bisabolol , Pentadecanoic acid , β-sitosterol glucoside and ursolic acid by column chromatography. Structures of the compounds have been established by spectral analysis.



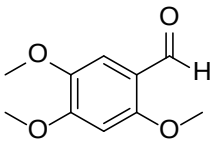
α-asarone



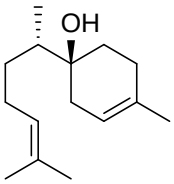
β-asarone



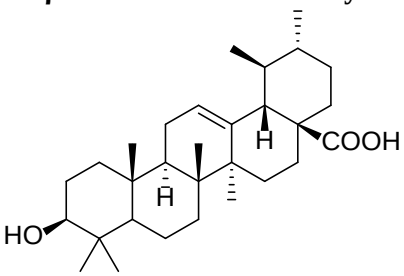
Methyl isoeugenol



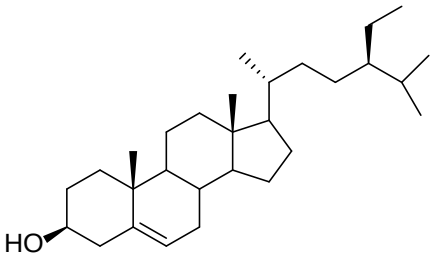
2,4,5-Trimethoxy benzaldehyde



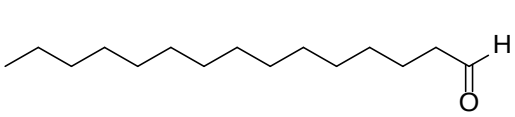
α-bisabolol



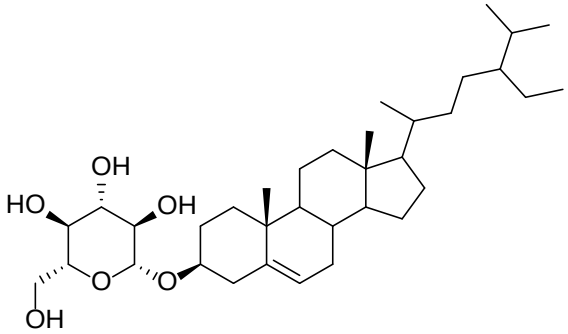
ursolic acid



β-sitosterol



Pentadecanoic acid



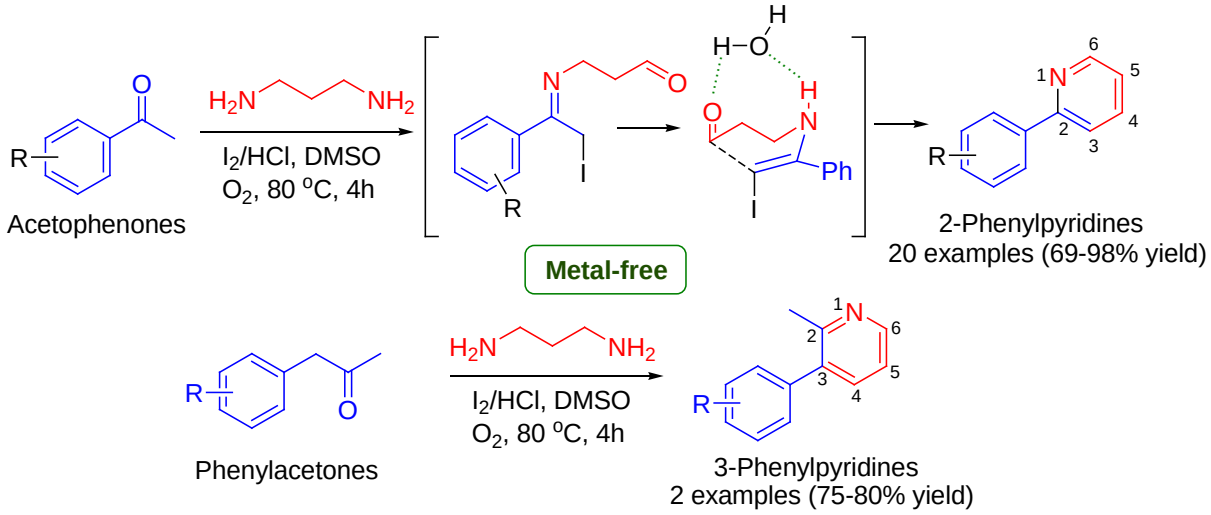
β-sitosterol glucoside

5.0 MEDICINAL CHEMISTRY

5.1 Metal-free oxidative cyclization of acetophenones with diamines: A facile access to phenylpyridines (*Chem. Commun*. 2016, 52, 1009-1012)

Sharma, R.; Patel, N.; Vishwakarma, R.A.; Bharatam, P.V.; Bharate, S.B.

An efficient metal-free access to 2- and 3-phenylpyridines via oxidative coupling of acetophenones or phenylacetones with 1,3-diaminopropane has been described. Reaction involves shorter reaction time, excellent yields and a broad substrate scope. Reaction proceeds via formation of imine, which further undergoes oxidative C-N bond cleavage, C-C bond formation and oxidation to give pyridine skeleton. The quantum chemical calculations identified the transition state for the reaction and helped in tracing reaction mechanism.

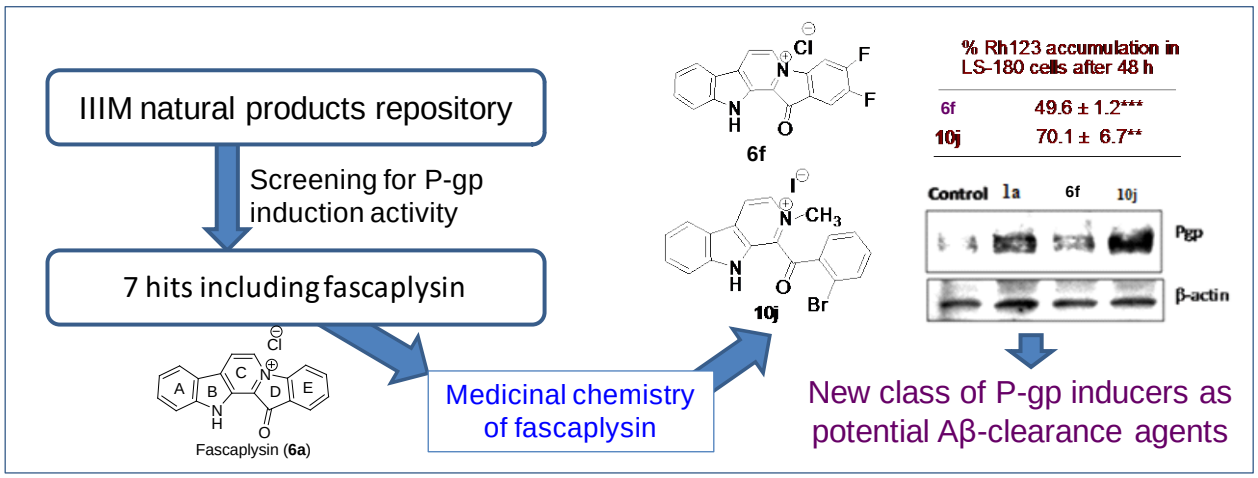


5.2 Discovery of a marine-derived bis-indole alkaloid fascaplysin, as a new class of potent P-glycoprotein inducer (*Eur. J. Med. Chem.* 2016, 107, 1–11)

Manda, S.; Sharma, S.; Wani, A.; Joshi, P.; Kumar, V.; Guru, S.K.; Bharate, S.S.; Bhushan, S.; Vishwakarma, R.A.; Kumar, A. and Bharate, S.B.

The screening of IIIM natural products repository for P-gp modulatory activity in P-gp over-expressing human adenocarcinoma LS-180 cells led to the identification of 7 natural products *viz*. withaferin, podophyllotoxin, 3-demethylcolchicine, agnuside, reserpine, seseberecine and fascaplysin as P-gp inducers. Fascaplysin, a marine-derived bis-indole alkaloid, was the most potent among all of them, showing induction of P-gp with EC_{50} value of 25 nM. P-gp induction is one of the recently targeted strategy to increase amyloid-β clearance from Alzheimer brains. Thus, we pursued a medicinal chemistry of fascaplysin to establish its structure-activity relationship for P-gp induction activity. Four series of analogs *viz*. substituted quaternary fascaplysin analogs, D-ring opened quaternary analogs, D-ring opened non-quaternary analogs, and *β*-carbolinium analogs were synthesized and screened for P-gp induction activity. Among the total of 48 analogs screened, only quaternary nitrogen containing analogs displayed promising P-gp induction activity; whereas non-planar non-quaternary analogs were devoid of this activity.

The P-gp induction activity of best compounds was then confirmed by western-blot analysis, which indicated that fascaplysin along with 4,5-difluoro analog of fascaplysin **6f** and D-ring opened analog **10j** displayed 4-8 fold increase in P-gp expression in LS-180 cells at 1 µM. Additionally, compounds **6a** and **6f** also showed inhibition of acetylcholinestease (AChE), an enzyme responsible for neuronal loss in Alzheimers disease. Thus, fascaplysin and its analogs showing promising P-gp induction along with AChE inhibition at 1 µM, with good safety window (LS-180: IC_{50} > 10 µM, hGF: 4 µM), clearly indicates their promise for development as an anti-Alzheimer agent.

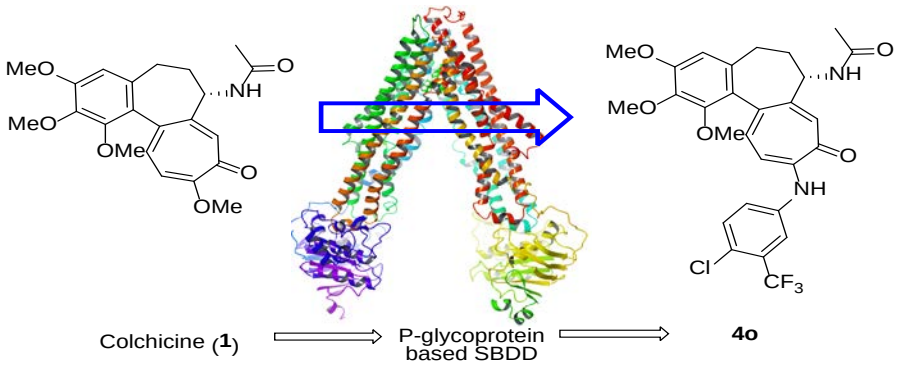


5.3 Design and synthesis of colchicine derivatives with potent *in vitro* and *in vivo* anticancer activity and reduced p-glycoprotein induction liability (*Org. Biomol. Chem*., 2015, 13, 5674-5689)

Singh, B.; Kumar, A.; Joshi, P.; Guru, S.K.; Kumar, S.; Wani, Z.A.; Mahajan, G.; Hussain, A.; Qazi, A.; Kumar, A.; Bharate, S.S.; Gupta, B.D.; Sharma, P.R.; Dar, A.H.; Saxena, A.K.; Mondhe, D.M.; Bhushan, S.; Bharate, S.B.; Vishwakarma, R.A.

Colchicine, a nature-derived microtubule polymerization inhibitor develops multi-drug resistance in tumor cells due to its P-gp substrate and induction activity, which in turn leads to its rapid efflux from tumor cells. This auto-induction of the efflux of colchicine remains a major challenge to medicinal chemists. Based on the structure-based molecular modeling, a series of new colchicine derivatives were designed and synthesized with a potential of reduced P-gp induction liability. Screening of prepared derivatives for P-gp induction activity revealed that number of derivatives possess remarkably lower P-gp-induction activity (>90% intracellular accumulation of rhodamine 123 in LS-180 cells) compared to the parent natural product colchicine (62% Rh123 accumulation in LS-180 cells). The reduced P-gp-induction activity of new derivatives may be due to their reduced ability to interact and change the conformation of P-gp. The synthesized derivatives were then screened for antiproliferative activity against two colon cancer cell lines including HCT-116 and Colo-205. The derivative **4o** showed potent cytotoxicity in HCT-116 cells with IC_{50} of 0.04 µM with significantly reduced P-gp induction liability. Compound **4o** also

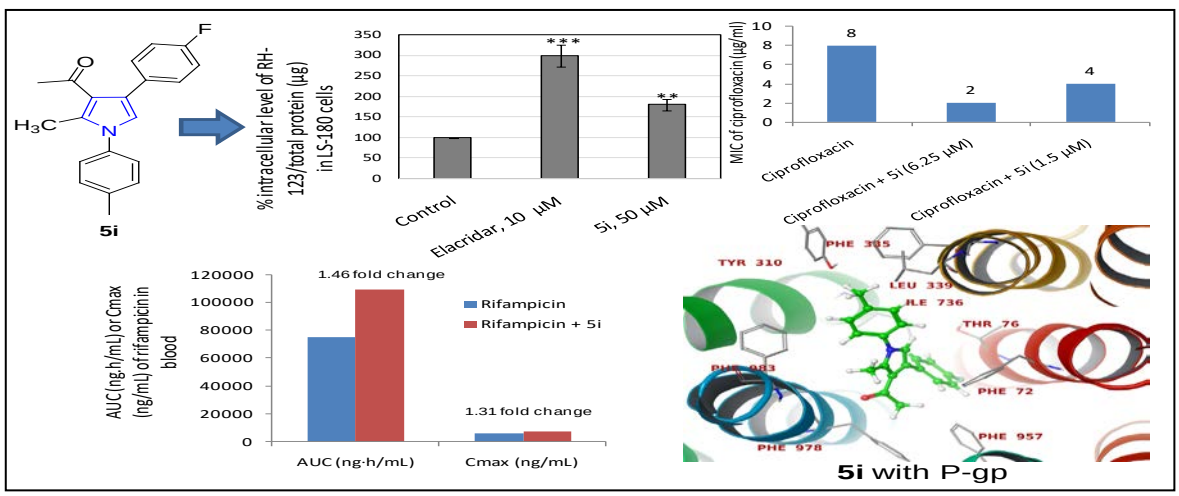
inhibited microtubule assembly and induced expression of pro-apoptotic protein p21. In Ehrlich solid tumor mice model, the compound **4o** at 2 mg/kg dose (oral) showed 38% TGI with no mortality. Compound **4o** possessing potent *in vitro* and *in vivo* anticancer activity, significantly reduced P-gp-induction activity and excellent physicochemical and pharmacokinetic properties opens up a new opportunity for colchicine scaffold.



5.4 Discovery of 4-acetyl-3-(4-fluorophenyl)-1-(p-tolyl)-5-methylpyrrole as a dual inhibitor of human P-glycoprotein and *Staphylococcus aureus* Nor A efflux pump (*Org. Biomol. Chem.*, 2015, 13, 5424-5431)

Jaideep B. Bharate, Samsher Singh, Abubakar Wani, Sadhana Sharma, Prashant Joshi, Inshad A. Khan, Ajay Kumar, Ram A. Vishwakarma, Sandip B. Bharate

Polysubstituted pyrrole natural products lamellarins are known to overcome multi-drug resistance in cancer via inhibition of p-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) efflux pumps. Herein, a series of simplied polysubstituted pyrroles, prepared via one-pot domino protocol, were screened for P-gp inhibition in P-gp overexpressing human adenocarcinoma LS-180 cells using rhodamine 123 efflux assay. Several compounds showed significant inhibition of P-gp at 50 µM, as indicated by increase in intracellular accumulation of Rh123 in LS-180 cells. Furthermore, pyrrole 5i decreased the efflux of digoxin, a FDA approved P-gp substrate in MDCK-MDR1 cells with IC_{50} of 11.2 µM. In in-vivo studies, following oral administration of a P-gp substrate drug rifampicin along with compound **5i**, the C_{max} and $AUC_{0-\infty}$ of rifampicin was enhanced by 31 and 46%. All compounds were then screened for their ability to potentiate ciprofloxacin

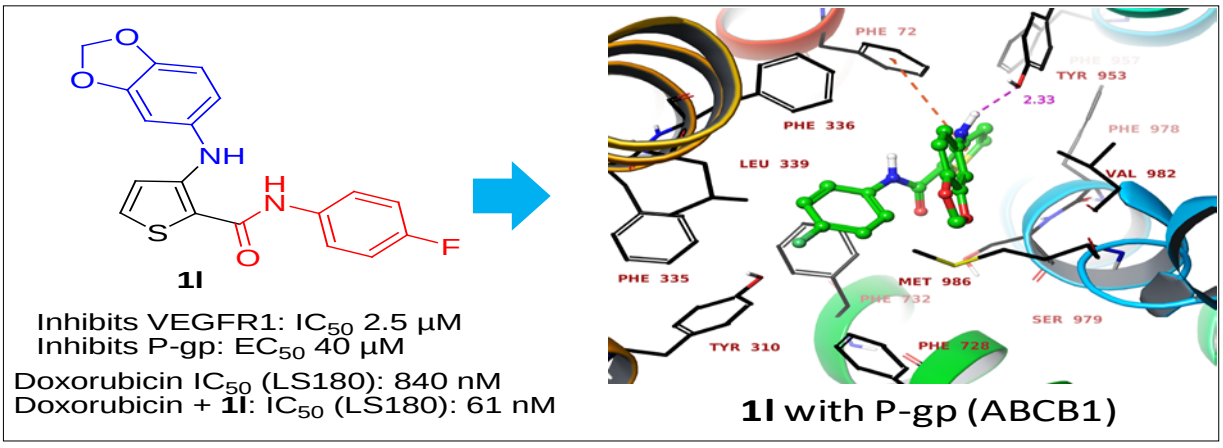


activity via inhibition of *Staphylococcus aureus* Nor A efflux pump. Pyrrole **5i** showed significant inhibition of *S. aureus* Nor A efflux pump with 8- and 4-fold reductions in the MIC of ciprofloxacin at 50 and 6.25 µM, respectively. The molecular docking studies of compound **5i** with the human P-gp and *S. aureus* Nor A efflux pump identified its plausible binding site and key interactions. Thus, the results presented herein strongly indicate the potential of this scaffold for use as multi-drug resistance reversal agents or bioavailability enhancers.

5.5 3-(Benzo[d][1,3]dioxol-5-ylamino)-N-(4-fluorophenyl) thiophene-2-arboxamide over comes cancer chemoresistance via inhibition of angiogenesis and P-glycoprotein efflux pump activity (*Org. Biomol. Chem.*, 2015, *13*, 4296-4309)

Ramesh Mudududdla, Santosh K. Guru, Abubakar Wani, Sadhana Sharma, Prashant Joshi, Ram A. Vishwakarma, Ajay Kumar, Shashi Bhushan, and Sandip B. Bharate

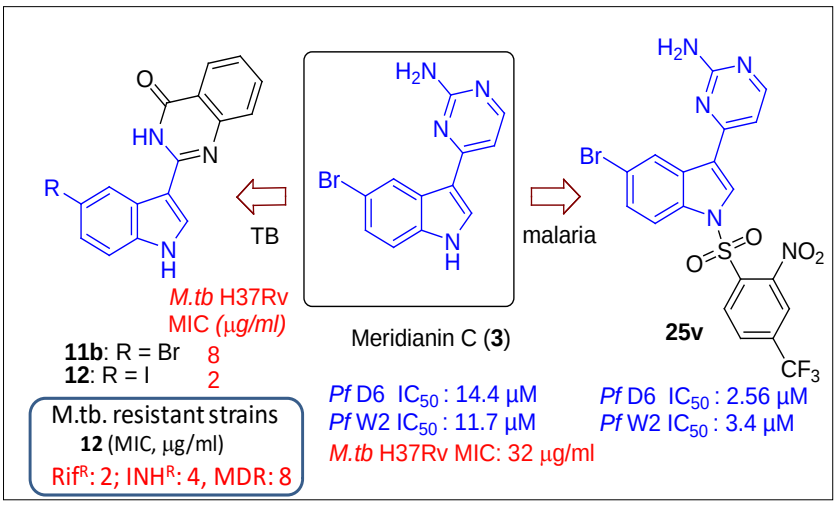
z3-((Quinolin-4-yl)methylamino)-N-(4-(trifluoromethoxy)phenyl)thiophene-2-carboxamide (OSI-930) is a potent inhibitor of c-kit and VEGFR2, currently under phase I clinical trials in patients with advanced solid tumors. In order to understand the structure-activity relationship, a series of 3-arylamino *N*-aryl thiophene 2-carboxamides were synthesized by modifications at both quinoline and amide domain of OSI-930 scaffold. All synthesized compounds were screened for in-vitro cytotoxicity in a panel of cancer cell lines and for VEGFR1 and VEGFR2 inhibition. Thiophene 2-carboxamides substituted with benzo[d][1,3]dioxol-5-yl and 2,3-dihydrobenzo[b][1,4]dioxin-6-yl groups **1l** and **1m** displayed inhibition of VEGFR1 with IC_{50} values of 2.5 and 1.9 µM, respectively. Compounds **1l** and **1m** also inhibited the VEGF-induced HUVEC cell migration, indicating its anti-angiogenic activity. OSI-930 along with compounds **1l** and **1m** showed inhibition of P-gp efflux pump (MDR1, ABCB1) with EC_{50} values in the range of 35-74 µM. The combination of these compounds with doxorubicin led to significant enhancement of the anticancer activity of doxorubicin in human colorectal carcinoma LS180 cells, which was evident by the improved IC_{50} of doxorubicin, increased activity of caspase-3 and significant reduction in colony formation ability of LS180 cells after treatment with doxorubicin. Compound **1l** showed 13.8-fold improvement in the IC_{50} of doxorubicin in LS180 cells. The ability of these compounds to possess dual inhibition of VEGFR and P-gp efflux pump demonstrates the promise of this scaffold for development as multi-drug resistance-reversal agents.



5.6 Synthesis, antimalarial and antitubercular activities of meridianin derivatives (*Eur. J. Med. Chem*. 2015, 98, 160-169)

Rammohan R. Yadav, Shabana I. Khan, Samsher Singh, Inshad A. Khan, Ram A. Vishwakarma and Sandip B. Bharate

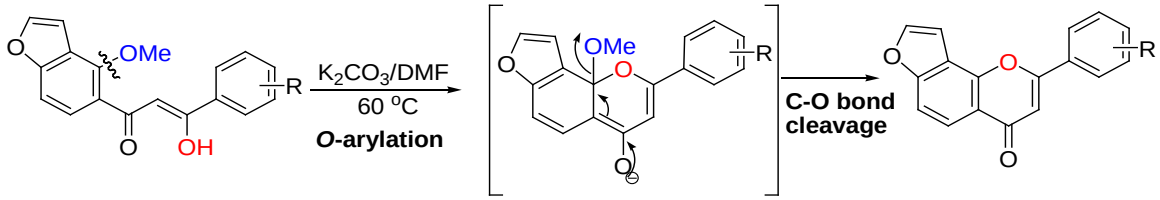
Meridianins are marine-derived indole alkaloids, known to possess kinase inhibitory and antimalarial activities. A series of *N*-aryl and heteroaryl sulfonamide derivatives of meridianins were prepared and screened for antimalarial activity against D6 and W2 strains of *Plasmodium falciparum*. 2-Nitro-4-trifluoromethyl sulfonamide derivative **25v** displayed promising antiplasmodial activity against both strains with IC_{50} values of 2.56 and 3.41 µM, respectively. These compounds were not cytotoxic to mammalian cell lines including VERO (monkey kidney fibroblasts), LLC-PK1 (pig kidney epithelial cells) and four cancer cell lines; SK-MEL (human malignant, melanoma), KB (human epidermal carcinoma), BT-549 (ductal carcinoma), SK-OV-3 (human ovary carcinoma) up to 25 µg/ml. Furthermore, all sulfonamide derivatives along with acyl, alkyl and C-ring modified derivatives of meridianins were screened for antitubercular activity against a sensitive strain ($H_{37}Rv$) of *Mycobacterium tuberculosis* (Mtb), wherein several compounds showed MIC values in the range of 5.2-304.8 µM. Meridianin C (**3**) and meridianin G (**7**) showed anti-tubercular activity with MIC values of 111.1 and 304.8 µM, respectively. The C-ring modified analog **12** exhibited potent anti-tubercular activity against $H_{37}Rv$ strain of Mtb with MIC of 5.2 µM. Furthermore, the most potent analogs **11b** and **12** were screened against two clinical isolates of *Mycobacterium tuberculosis* INH^R and MDR and one laboratory generated mutant strain Rif^R. These two analogs **11b** and **12** displayed promising activity against these resistant strains with MIC values in the range of 5.2-187.7 µM. This is the first report on the anti-tubercular activity of this scaffold.



5.7 An efficient transformation of furano-hydroxychalcones to furanoflavones via base mediated intramolecular tandem *O*-arylation and C-O bond cleavage: A new approach for synthesis of furanoflavones (*Org. Biomol. Chem.*, 2015, *13*, 10461-10465)

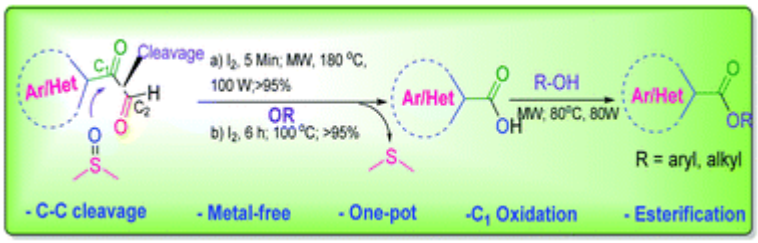
Sharma, Rajni; Vishwakarma, R.A.; Bharate, S.B.

A new and efficient potassium carbonate mediated intramolecular tandem *O*-arylation followed by C-O bond cleavage of furano-hydroxychalcones has been described. The treatment of furano-hydroxychalcones pongamol and ovalitenone with potassium carbonate in DMF led to the direct formation of furanoflavones lanceolatin B and pongaglabrone in excellent yields. This is the first report on cyclization of furano-hydroxychalcones *via* C-O bond cleavage (demethoxylation) to produce furanoflavonoids.



5.8 DMSO/I_2 mediated C–C bond cleavage of α-ketoaldehydes followed by C–O bond formation: A metal-free approach for one-pot esterification

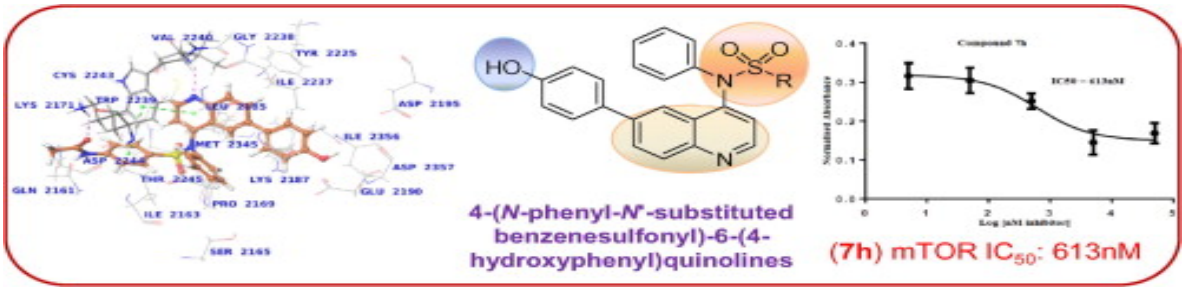
V. Venkateswarlu; K. A. Aravinda Kumar; S. Gupta; D. Singh; R.A. Vishwakarma; S. D.Sawant



One-pot I_2/DMSO mediated metal-free C–C bond cleavage of aryl-/heteroaryl- or aliphatic α-ketoaldehydes offering a carboxylic acid followed by esterification is presented. A novel and efficient I_2/DMSO mediated metal-free strategy is presented for the direct C–C bond cleavage of aryl-/heteroaryl- or aliphatic α-ketoaldehydes by C_2-decarbonylation and C_1-carbonyl oxidation to give the corresponding carboxylic acids followed by esterification in one pot, offering excellent yields in both the steps. Here, DMSO acts as the oxygen source/oxidant and this reaction works very well under both conventional heating and microwave irradiation. This is a very simple and convenient protocol.

5.9 4-(*N*-phenyl-*N*'-substituted benzenesulfonyl)-6-(4-hydroxyphenyl) quinolines as Inhibitors of Mammalian Target of Rapamycin

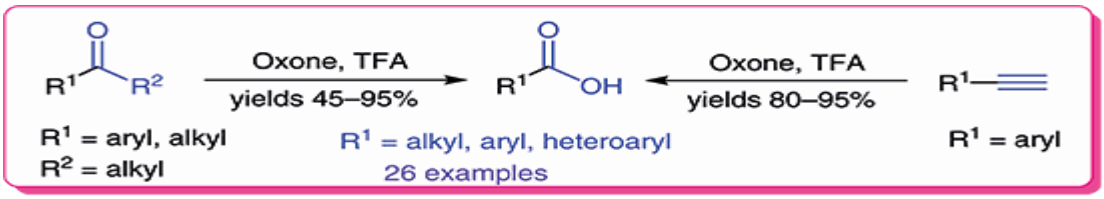
Venkateswarlu, V.; Pathania, A. S.; K.A. Aravinda Kumar; P. Mahajan,; A. Nargotra; R. A. Vishwakarma; F.A. Malik; S.D Sawant



A series of 4-(*N*-phenyl-*N*′-substituted benzenesulfonyl)-6-(4-hydroxyphenyl) quinolines was designed, synthesized and evaluated for their biological potential as anticancer agents by screening the molecules against panel of five human cancer cell lines viz. HL-60, MiaPaCa-2, HCT116, PC-3 and HEP-G2. The series has shown good mTOR inhibitory activity at 0.5 µM concentration. The representative compound **7h** was found to be most active with the IC_{50} of 613 nM against mTOR. In supportive evidence, the western blotting experiment revealed that compound **7h** is more potent in inhibiting p-mTOR (S2448) activity in 2–4 h at 5 and 10 µM concentrations and was selective and specific towards mTORC1 versus mTORC2. Towards understanding the mechanistic aspects we studied cell cycle analysis, mitochondrial membrane potential loss in MiaPaca-2 cells for compound **7h**. The docking study for this series was performed to understand the binding mode of the compounds and its consequent effect in biological activity, the initial interaction studies were found to be useful in design of molecules, where compound **7h** has shown additional H-bond interaction with Lys2171 apart from Val2240 and also a small hydrophobic cleft was observed with Leu2185, Met2345 and Ile2356.

5.10 A metal-free approach to carboxylic acids by oxidation of alkyl, aryl or heteroaryl alkyl ketones and arylalkynes

K. A. A. Kumar; V. Venkateswarlu; R. A. Vishwakarma,; *S.D. Sawant*

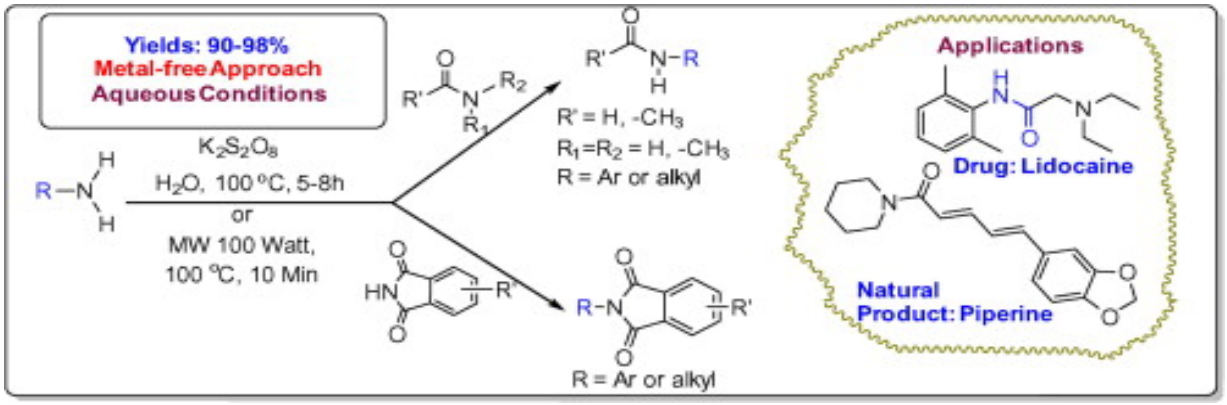


The metal-free oxidation of dialkyl, alkyl aryl, or alkyl heteroaryl ketones or arylalkynes to the corresponding carboxylic acids is achieved using an oxidative mixture of Oxone and trifluoroacetic acid. This green method is a simple and mild protocol to obtain carboxylic derivatives in excellent yields.

5.11 A metal-free approach for transamidation of amides with amines in aqueous media

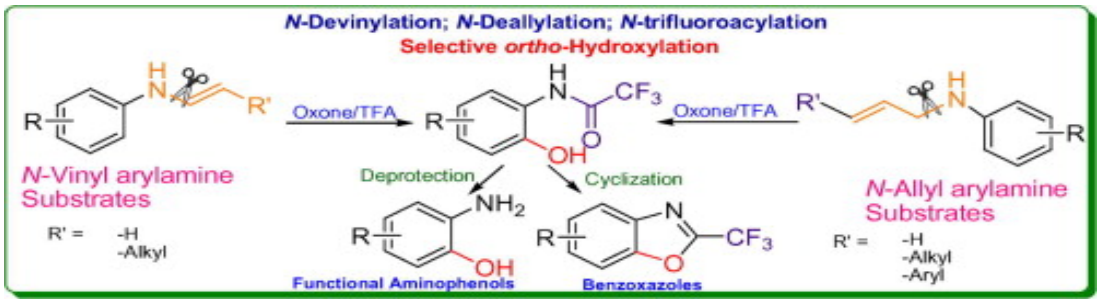
Srinivas, M.; Hudwekar, A. D.; Venkateswarlu, V.; Reddy, G. L.; Aravinda Kumar, K. A.; Vishwakarma, R. A.; S. D. Sawant

An efficient, environmentally benign and a mild protocol for transamidation of amides with a variety of amines in the presence of $K_2S_2O_8$ using stoichiometric quantity in aqueous conditions has been established. This method works under conventional thermal conditions and in microwave irradiation as well. A series of amides have been prepared using this reaction and this is a greener protocol for transamidation, which offers a diverse kind of substrate scope with exclusive product formation (yields 90–98%).



5.12 Direct C–N bond cleavage of N-vinyl or *N*-allyl arylamines: A metal-free strategy for *N*-devinylation and *N*-deallylation

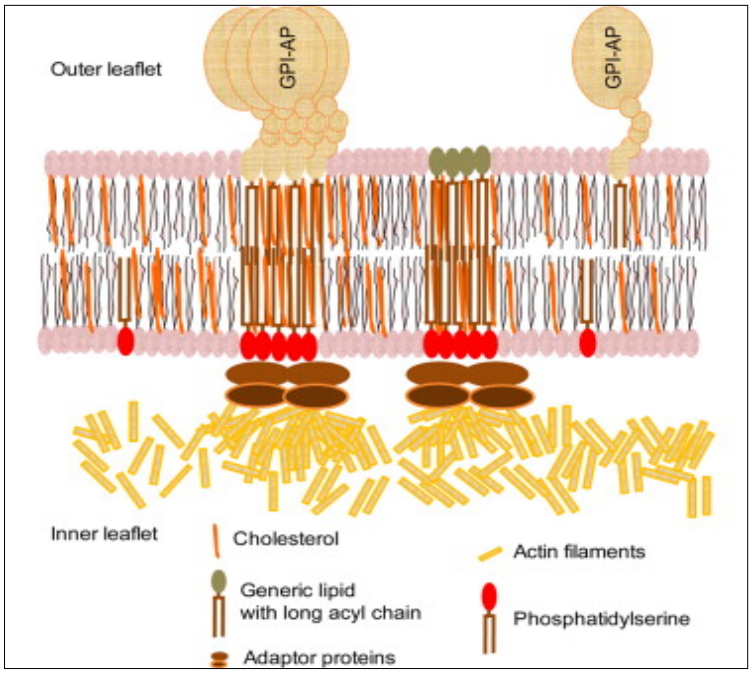
S. Balgotra, V. Venkateswarlu, R. A. Vishwakarma, *S. D. Sawant*



A simple and convenient N-devinylation and N-deallylation strategy for *N*-vinyl and *N*-allyl arylamines in the presence of TFA/oxone is presented with the formation of selective *ortho*-hydroxylated and *N*-trifluoroacylated arylamine product in good yields. This method is important for protection/deprotection of arylamines in organic synthesis and useful as a medicinal chemistry tool at late stage modifications in drug discovery programs.

5.13 Transbilayer Lipid Interactions Mediate Nanoclustering of Lipid-Anchored Proteins

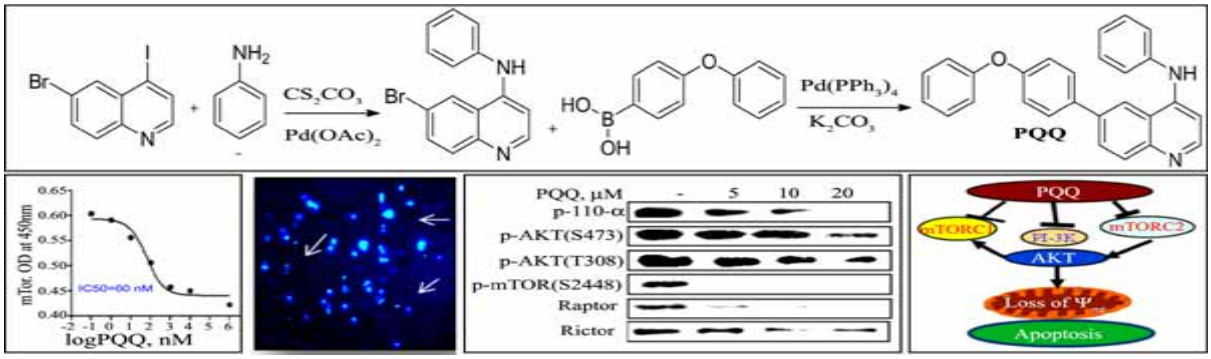
R. Raghupathy, A. Ambika, A. Polley, P. P. Singh, M. Yadav, C. Johnson, S. Suryawanshi, V. Saikam, S. D. Sawant, A. Panda, Z. Guo, R. A. Vishwakarma, M. Rao, S. Mayor



Understanding how functional lipid domains in live cell membranes are generated has posed a challenge. Here, we show that transbilayer interactions are necessary for the generation of cholesterol-dependent nanoclusters of GPI-anchored proteins mediated by membrane-adjacent dynamic actin filaments. We find that long saturated acyl-chains are required for forming GPI-anchor nanoclusters. Simultaneously, at the inner leaflet, long acyl-chain-containing phosphatidylserine (PS) is necessary for transbilayer coupling. All-atom molecular dynamics simulations of asymmetric multicomponent-membrane bilayers in a mixed phase provide evidence that immobilization of long saturated acyl-chain lipids at either leaflet stabilizes cholesterol-dependent transbilayer interactions forming local domains with characteristics similar to a liquid-ordered (*lo*) phase. This is verified by experiments wherein immobilization of long acyl-chain lipids at one leaflet effects transbilayer interactions of corresponding lipids at the opposite leaflet. This suggests a general mechanism for the generation and stabilization of nanoscale cholesterol-dependent and actin-mediated lipid clusters in live cell membranes.

5.14 A novel quinoline based second-generation mTOR inhibitor that induces apoptosis and disrupts PI3K-Akt-mTOR signaling in human leukemia HL-60 cells

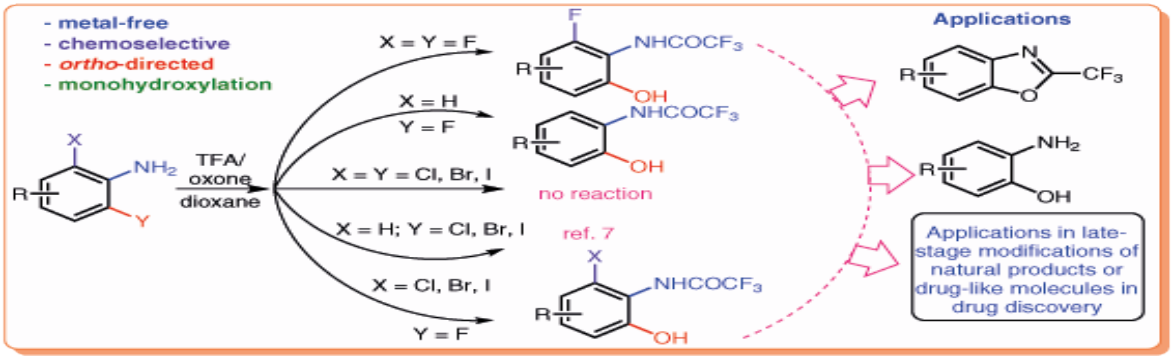
S. K Guru, V. Venkateswarlu, F. A. Malik, S. D. Sawant, R.A. Vishwakarma, S. Bhushan



Deregulation of the PI3K-Akt-mTOR pathway is unanimously pragmatic in a number of tumors. This pathway pedals proliferation, survival, translation, and coupled with tumorassociated endurance. Current efforts focus on the discovery and development of novel inhibitors of this pathway. We have discovered6-(4-phenoxyphenyl)-N-phenylquinolin-4-amine [PQQ] as a potent mTOR inhibitor with IC50 value of 64nM in a cell-based and cell-free mTOR assay. Mechanistically, PQQ was found to be a strong PI3K-Akt-mTOR-p70S6K cascade inhibitor in Human promyelocytic leukemia HL-60 cells. Moreover, it was found to be dual mTORC1 and mTORC2 inhibitor that inhibit the entire mTOR kinase-dependent functions and feedback commencement of PI3K/Akt pathway. PQQ simultaneously induces apoptosis *via* mitochondrial dependant pathway, which was confirmed through a battery of the assays, e.g. cellular and nuclear microscopy, annexin-V assay, cell cycle analysis and loss of mitochondrial membrane potential. In summary, PQQ discovered as a novel secondgeneration mTOR inhibitor with significant cytotoxic and apoptotic potentials. Thus, it might be a significant lead structure for the development of mTOR-targeted based anti-cancer therapeutics.

5.15 Metal-free Chemoselective *ortho*-C(sp^2)–F Bond Hydroxylation and *N*-trifluoroacylation of Fluoroarylamines for Domino Synthesis of *N*-trifluoroacyl-*ortho*-aminophenols

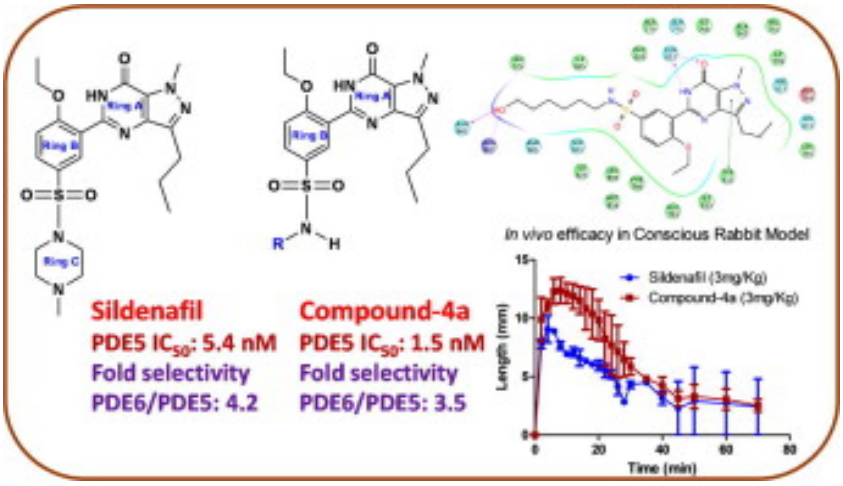
V. Venkateswarlu, S. Balgotra, R. A. Vishwakarma, S. D. Sawant



A novel chemoselective reaction for the formation of C–O bonds by C(sp^2)–F bond cleavage and concomitant N-trifluoroacylation of fluoroanilines using trifluoroacetic acid and Oxone® is presented. This domino reaction gives *o*-hydroxy-*N*-trifluoroacetanilides in good yields under metal-free conditions in a single step. Selective *ortho*-directed monohydroxylation and N-trifluoroacylation of 2- and 6-fluoro- or 2,6-difluoro-substituted anilines takes place in this transformation.

5.16 Discovery of Novel Pyrazolopyrimidinone Analogs as Potent Inhibitors of Phosphodiesterase Type-5

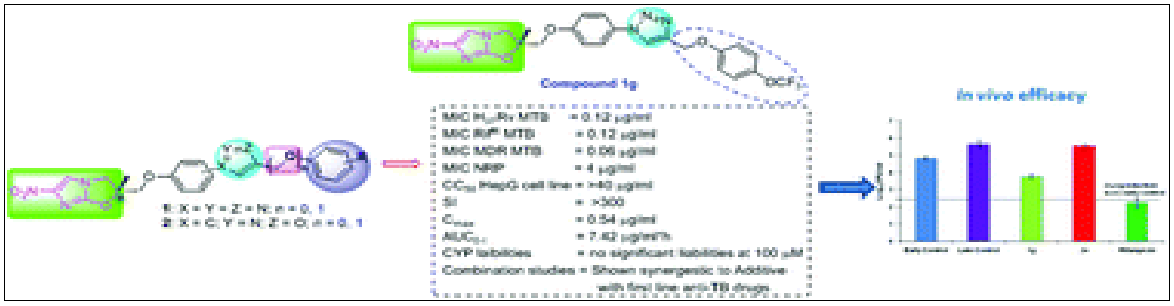
S. D. Sawant, G.L. Reddy, M. Ishaq Dar, M. Srinivas, G. Gupta, P. K. Sahu, P. Mahajan, S. Singh, S.C. Sharma, M. Tikoo, G. D. Singh, A. Nargotra, R. A. Vishwakarma, S. H. Syed



Cyclic guanosine monophosphate (cGMP) specific phosphodiesterase type-5 (PDE5), a clinically proven target to treat erectile dysfunction and diseases associated with lower cGMP levels in humans, is present in corpus cavernosum, heart, lung, platelets, prostate, urethra, bladder, liver, brain, and stomach. Sildenafil, vardenafil, tadalafil and avanafil are FDA approved drugs in market as PDE5 inhibitors for treating erectile dysfunction. In the present study a lead molecule 4-ethoxy-*N*-(6-hydroxyhexyl)-3-(1-methyl-7-oxo-3-propyl-6,7-dihydro-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)benzenesulfonamide, that is, compound-**4a**, an analog of pyrazolopyrimidinone scaffold has been identified as selective PDE5 inhibitor. A series of compounds was synthesized by replacing *N*-methylpiperazine moiety (ring-C) of sildenafil structure with different *N*-substitutions towards sulfonamide end. Compound-**4a** showed lower IC_{50} value (1.5 nM) against PDE5 than parent sildenafil (5.6 nM) in in vitro enzyme assay. The isoform selectivity of the compound-**4a** against other PDE isoforms was similar to that of the Sildenafil. In corroboration with the in vitro data, this molecule showed better efficacy in in vivo studies using the conscious rabbit model. Also compound-**4a** exhibited good physicochemical properties like solubility, caco-2 permeability, *c* Log *P* along with optimal PK profile having no significant CYP enzyme inhibitory liabilities. Discovery of these novel bioactive compounds may open a new alternative for developing novel preclinical candidates based on this drugable scaffold.

5.17 Synthesis of new generation triazolyl and isoxazolyl containing 6-nitro-2,3-dihydroimidazooxazoles as anti-TB agents: In vitro, Structure-activity relationship, pharmacokinetics and in vivo evaluation

G. Mungala, K. Reddy Yempala, S. Singh, S. Sharma, N.P. Kalia, V.K. Singh, S. Kumar, S. D. Sawant, I. A. Khan, R.A. Vishwakarma, P. P. Singh



Promising nitroimidazoloxazole scaffold gives another novel triazolyl-containing 6-nitro-2,3-dihydroimidazooxazole as anti-TB lead. The nitroimidazole scaffold has attracted great interest in the last decade, which ultimately led to the discovery of the successful drug Delamanid for multi-drug resistant tuberculosis (MDR-TB). Herein, we report medicinal chemistry on a 6-nitro-2,3-dihydroimidazooxazole (NHIO) scaffold with SAR on the novel series of triazolyl- and isoxazolyl-based NHIO compounds. In the present study, 41 novel triazolyl- and isoxazolyl-based NHIO compounds were synthesized and evaluated against *Mycobacterium tuberculosis* (MTB) $H_{37}Rv$. The active compounds with MIC of 0.57–0.13 μM were further screened against dormant, as well as against resistant strains of MTB. Based on the overall *in vitro* profile, five compounds were studied for *in vivo* oral pharmacokinetics, wherein two compounds: **1g** and **2e** showed a good PK profile. In *in vivo*efficacy studies in the intra-nasal model of acute infection, **1g** showed 1.8 and 1 log CFU reduction with respect to the untreated and early control, respectively. The lead compound **1g**also showed an additive to synergistic effect in combination studies with first line-TB drugs and no CYP inhibition. From the present studies, the compound **1g** represents another alternative lead candidate in this class and needs further detailed investigation.

6.0 FERMENTATION DIVISION

6.1 Production of nonribosomal peptides by psychrotrophic fungus: *Trichoderma velutinum* ACR-P1

Trichoderma is an anamorphic filamentous fungal genus with immense potential for production of small valuable secondary metabolites with indispensable biological activities. Microbial dynamics of a psychrotrophic strain *Trichoderma velutinum* ACR-P1, isolated from unexplored niches of the Shiwalik region, bestowed with rich biodiversity of microflora, was investigated for production of non ribosomal peptides (NRPs) by metabolite profiling by Intact-Cell Mass Spectrometry (ICMS) employing Matrix Assisted Laser Desorption/Ionisation-Time Of Flight (MALDI-TOF) mass spectrometer. The fungus used in the current study was isolated from the soil sample collected from cold habitat of the Shiwalik hills at about 2,500 ft from the sea level, Jammu and Kashmir, India, during extreme winters. Temperature of the area, during extreme winters usually remains in range from −5 to 2 °C. The fungus was isolated on dextrin peptone agar (DPA) medium and maintained on the same medium in slants at 4 °C. **ICMS studies by MALDI-TOF mass spectrometer** A few micrograms of fungal mycelia were scraped carefully from agar petri plates avoiding any media interference and suspended in acetonitrile/water (7:3 v/v). Ten microliters of the suspension was premixed with 10 µl matrix solution in varied ratio of 1:1, 2:1, 5:1, or 25:1. (5 or 10 mg of CHCA or DHB in 1 ml of 70:30 acetonitrile: water v/v) vortexed and centrifuged at 5000×g for 20 min. One microliter of mixture was directly spotted onto target wells of 364-well sample plate either using premixed two-layer volume technique or dried droplet method and air-dried prior to analysisMass spectra were obtained on Applied Biosystems 4800 MALDI-TOF/TOF mass analyzer. Mass accuracy specification was kept at ±0.05 Da.

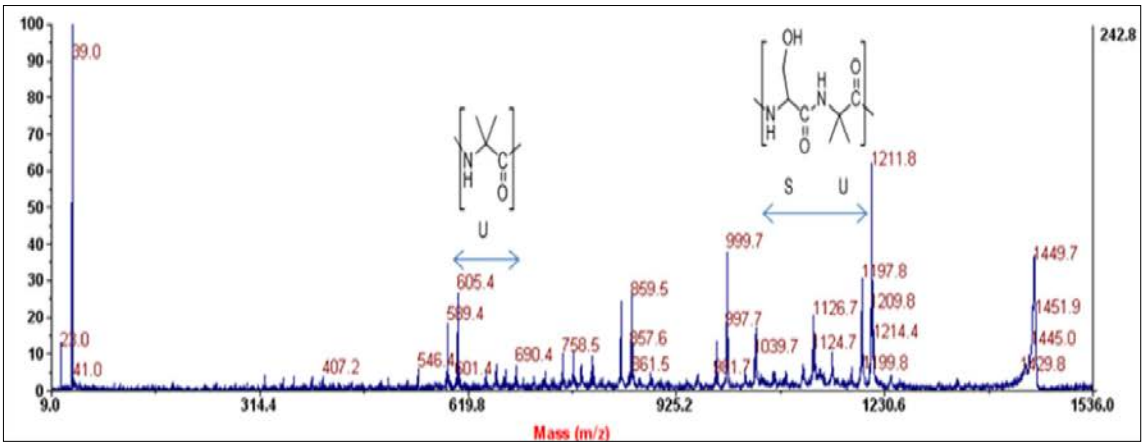


Figure 6.1.1: MS spectrum of *T. velutinum* ACR-P1

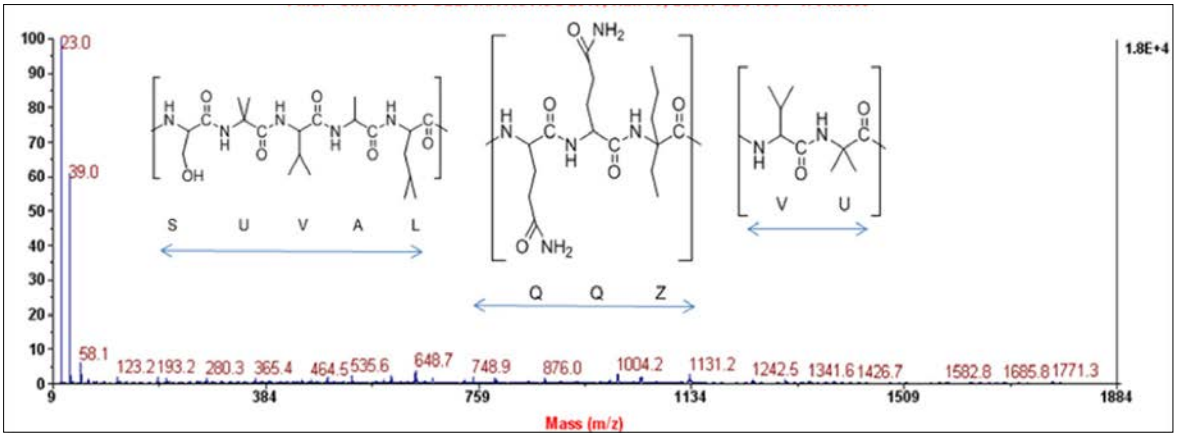


Figure 6.1.2: MS/MS spectra of *T. velutinum* ACR-P1 confirming nonribosomal peptides

Being the first report on NRPs production by *T. velutinum*, studies on optimization of growth conditions by Response Surface Methodology (RSM) for production of NRPs by ACR-P1 was carried out strategically. Multifold enhancement in the yield of NRPs belonging to subfamily SF4 with medium chain of amino acid residues having *m/z* 1437.9, 1453.9, 1452.0 at pH 5.9 at 20°C and of subfamily SF1with long chain amino acid residues having *m/z* 1770.2, 1784.2, 1800.1, 1802.1, 1815.1 was achieved at pH 7.0 at 25°C. Complexities of natural mixtures were thus considerably reduced under respective optimized culture conditions accelerating the production of novel microbial natural products by saving time and resources.

Optimization study for peptide production through RSM approach

Optimization study for peptide production through RSM approach CCD approach was adopted for investigating the individual and interactive effects of the selected culture conditions. The statistical program generated 13 random experiments for the two respective metabolites each, being four factorial, four axial, and five central. The ICMS results were studied in terms of relative % intensity of respective secondary metabolites. All the experiments were conducted according to the CCD matrix at random, to avoid the possibility of any systematic errors in measurements. The experiments were carried out in random order with five replicates at the central point to calculate the pure error of the model. Result of four factorial experiments for SF4 showed that at pH 5.0, at two different temperatures of 10 and 30°C favored SF4 formation. At higher temperature of 30°C, formation of other undesired metabolites was observed at trace levels. Combination of pH 7.0 at both low and high temperatures of 10 and 30°C did not even favor SF4 formation comparatively as it does to SF1 corroborating the thought that neutral pH is not favorable to SF4 group even at broad temperature ranges. Four axial experiments with slightly acidic to acidic conditions supported the formation of SF4 group as compared to SF1 group. It was also observed that at slight acidic conditions, lower temperature range were yielding increased production of SF4 (pH 5.0 at 10^{o} C).

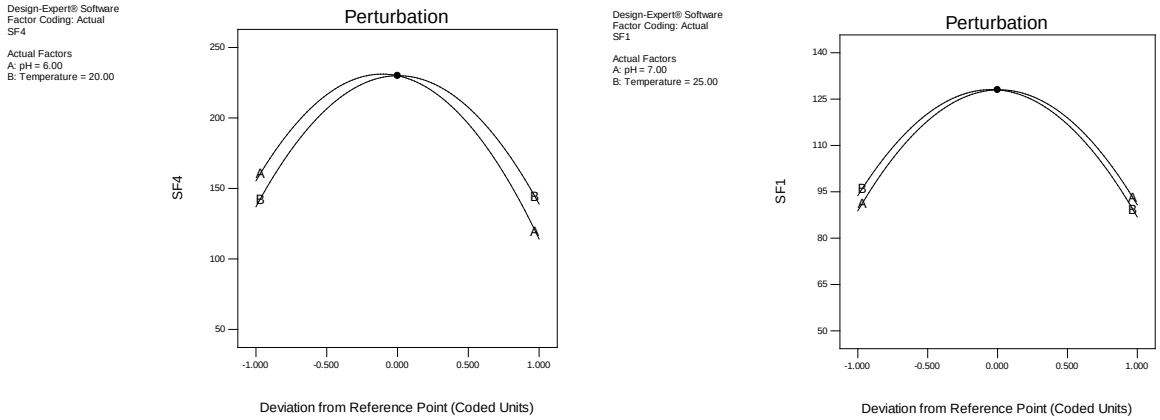


Figure 6.1.3: Perturbation plots showing deviation from reference point for production of SF4 and SF1, respectively

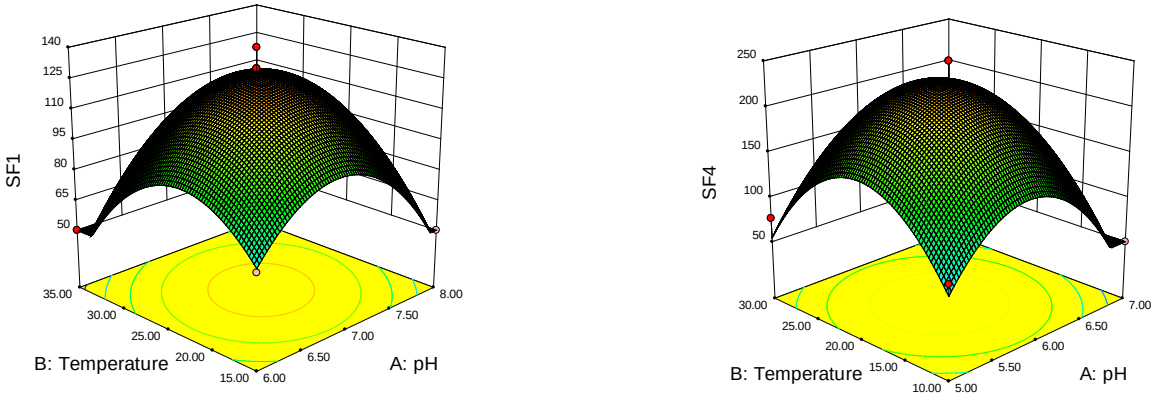


Figure 6.1.4: 3D response surface graphs for interaction of pH (A) and temperature (B) for the production of SF4 and SF1 by *T. velutinum* ACR-P1

Optimization of these interactive effects resulted in an exciting observation that culture conditions of pH 5.9 at 20 °C were optimum for the production of SF4 group, which resulted in multifold quantitative increase, compared to standard growth conditions (pH 7.0 and temperature 30 °C). Similarly, other condition of pH 7.0 at 25 °C supported maximum production of SF1 group with multifold quantitative increase. It is important here to mention that two different conditions were optimized for fungal strain *T. velutinum* ACR-P1 for two different groups of nonribosomal peptides. The results obtained after optimization studies by RSM were verified by conducting the experiments in triplicates under the optimized conditions. The experimental values of the triplicates were compared with the predicted val.ues of the metabolites. The experimental values obtained were in close agreement with the predicted values of the developed models with acceptable errors

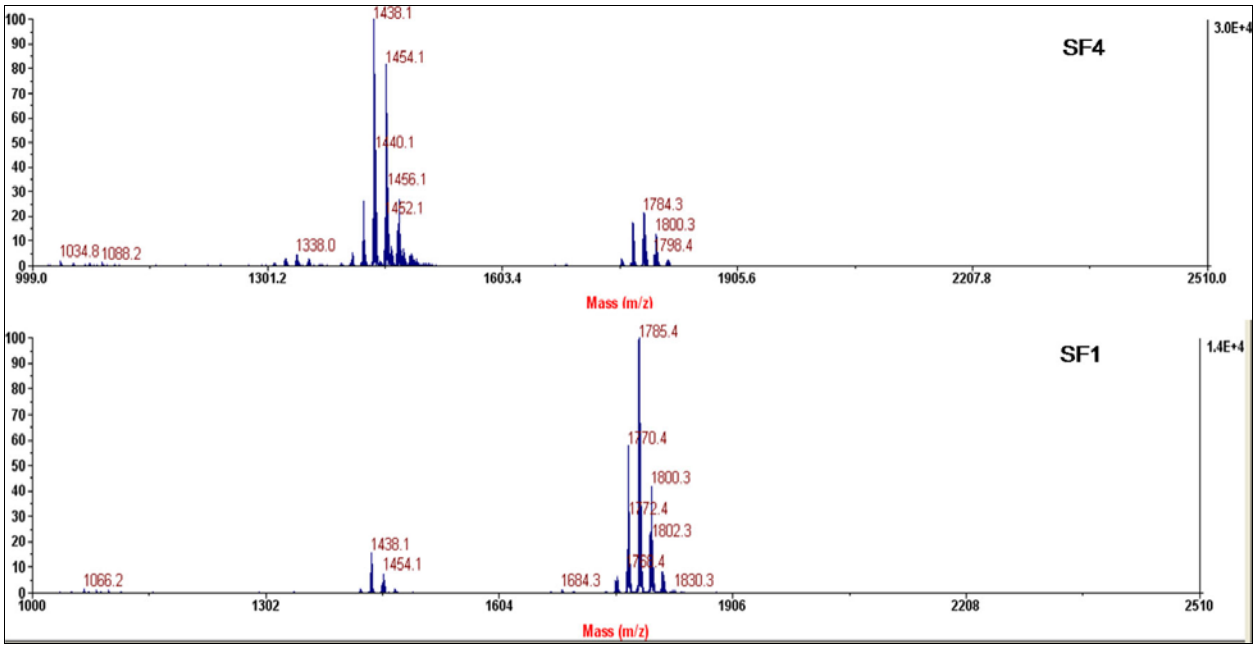


Figure 6.1.5 Validation of RSM model in *T. velutinum* ACR-P1 for production of the SF1 and SF4 peptides after optimization

The strategic integration of optimization studies for the respective peptides SF4 and SF1 by RSM methodology with ICMS technique have led to significant reduction in time as compared to tedious, labour intensive work required to know the best suitable condition for the production of metabolites in a highly efficient and simplified manner from a new and unexploited taxon of *Trichoderma*. Study also threw light on how different growth conditions for *T.velutinum* ACR-P1 can reduce complexities in natural mixtures giving a desirable advantage in the tedious process of purification and characterization being conducted for isolation and structural elucidation of the respective microbial peptides in our future research.

6.2 Epigenetic modifier induced alteration in the secondary metabolic profile of *Aspergillus fumigatus* (GA-L7): an endophytic fungus from *Grewia asiatica* L.

The effect of epigenetic modifier on the metabolic profile of *Aspergillus fumigatus* (GA-L7), an endophytic fungus from *Grewia asiatica* was studied. Addition of an epigenetic modifier i.e valproic acid in the growth medium, resulted in the alteration of secondary metabolic profile of *Aspergillus fumigatus* (GA-L7) and enhancement of a metabolite fumiquinazoline C by 10 folds which was produced in traces in the control untreated culture. Fumiquinazolines are signature peptidyl alkaloids and have been found to have significant antibacterial, antifungal and antitumour properties. The effect of epigenetic modifiers was studied in shake flasks and 500 µM of the following epigenetic modifiers were used namely: valproic acid, sodium butyrate and suberoylanilide hydroxamic acid (SAHA). 500 µM of the epigenetic modifier was added to 100 ml PDB in 250 ml Erlenmeyer flasks. The flasks were then inoculated with 2% seed of 2 days old seed of GA-L7. Also a control was run altogether with the above flasks containing only potato dextrose broth and inoculated with the same seed culture. After incubating the flasks for 8 days, all the flasks were terminated followed by extraction as previously described. All the extracts

were evaluated using LC-ESI-MS and also their TLC pattern was compared to control. The extracts were also evaluated for antimicrobial activity against a panel of pathogens. For quantification, LCMS-grade acetonitrile, water and formic acid, used in the study, were purchased from Merck, Germany. Isolated compounds (pseurotin A, pseurotin D , pseurotin F_2 , fumagillin , tryprostatin C , gliotoxin, and bis (dimethylthio)gliotoxin) were used as standard compounds. Purity of all the investigated compounds was confirmed by HPLC and all of them were ≥98.50 %.

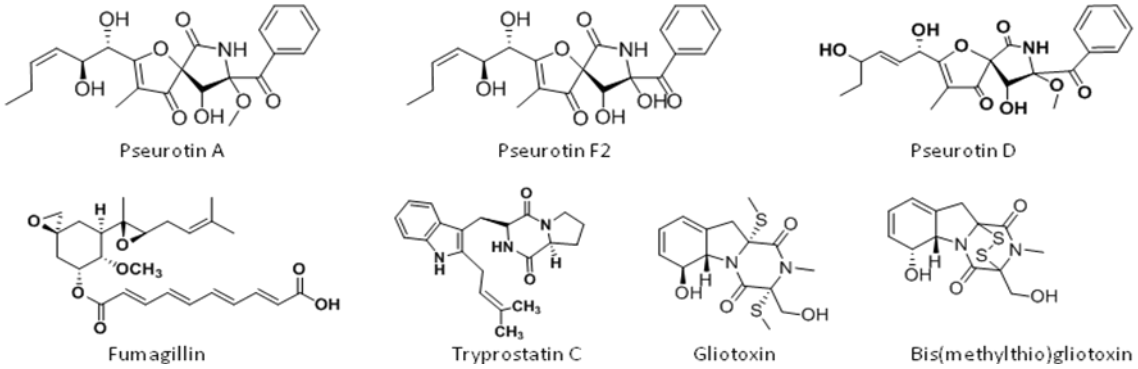


Figure 6.2.1 : Structures of the compounds isolated from A. fumigates (GA-L7)

Comparative profiling with LC-ESI-MS and TLC demonstrated that cultures treated with SAHA, sodium butyrate and 5-azacytidine did not produce new or enhanced secondary metabolite profile. Valproic acid treated *Aspergillus fumigatus* (GA-L7) showed an altered metabolic profile. LC-ESI-MS spectra of valproic acid treated fungal culture showed significant enhancement in the production of a secondary metabolite, fumiquinazoline C which was earlier present in minute quantities in the crude extract. Fumiquinazoline C was then isolated from the valproic acid treated extract of *Aspergillus fumigatus* (GA-L7).

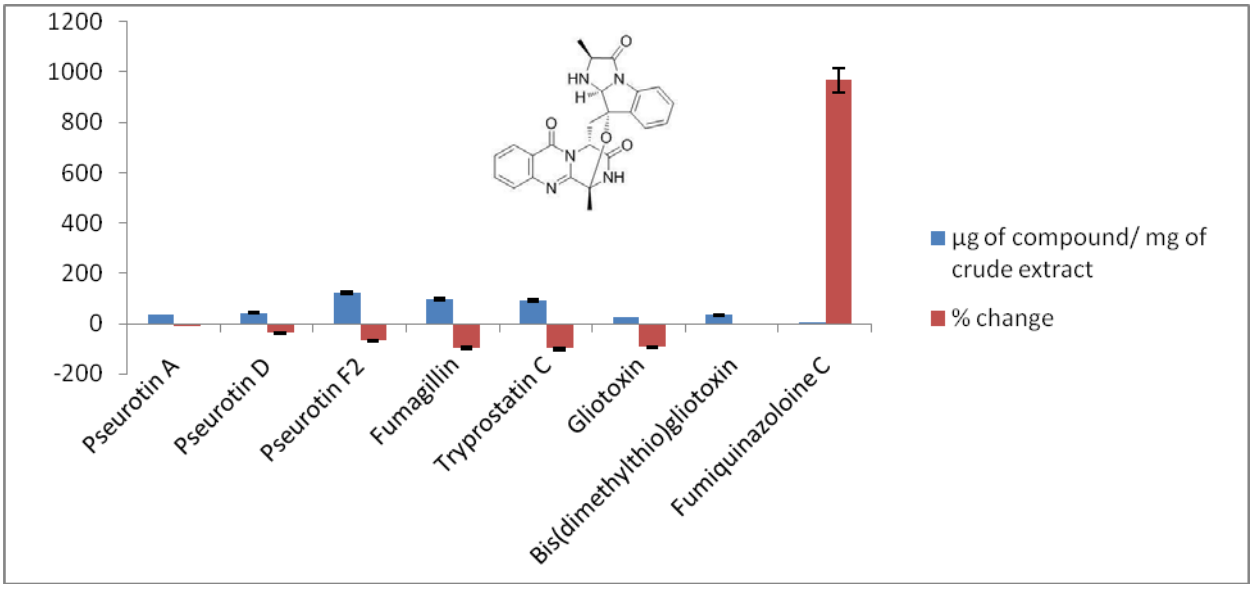
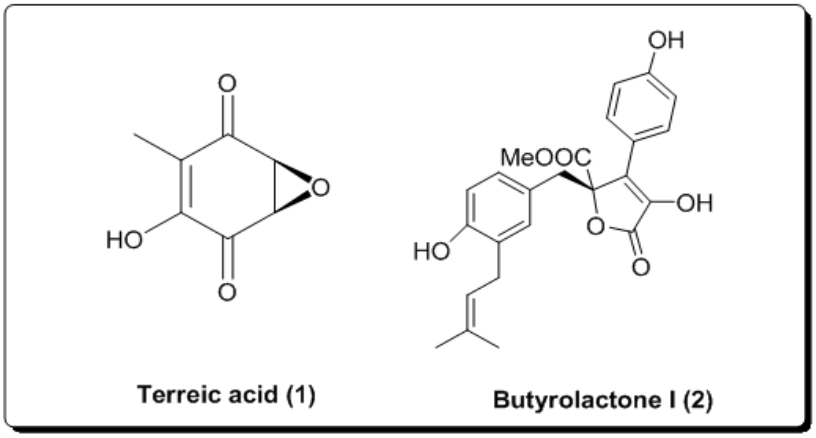


Figure 6.2.3 : Quantification of compounds in extract prepared from valproic acid treated culture with respect to compounds present in crude extract of untreated control culture

6.3 Isolation of secondary metabolites from *Aspergillus terreus*



The fungal strain *Aspergillus terreus* (MRCJ-356), was isolated from a soil sample collected from Shiwalik region situated at a height of about 900 meters from sea level, having geographical location 33.38^{o}N 74.38^{o}E. Seed inoculum was prepared in Sabouard dextrose broth (SDB) medium. The seed culture was allowed to grow at 28^{o}C with shaking at 200 rpm for a period of two days. Batch fermentation was carried out in a 7L fermentor (New Brunswick Scientific BioFlo 110) having 5L of SDB medium under following conditions: Temperature 28° C; agitation speed 200 rpm and aeration 0.5 vvm. Fermentation was carried out for 10 days and batch was terminated and processed for isolation of desired compound. The ethyl acetate extract (100 mg) was obtained from the liquid fermentation broth and subjected to column chromatography on Silica Gel 60 using a stepwise gradient from n-hexane: EtOAc 90:10 v/v (Compound 1, 18 mg); n-hexane: EtOAc 70:30 v/v (Butyrolactone 2, 22 mg). Both secondary metabolites isolated were screened for screen its activity against the acetyltransferase domain of a bifunctional enzyme, *Escherichia coli N*-acetylglucosamine-1-phosphate-uridyltransferase/glucosamine-1-phosphate-acetyltransferase (GlmU). Terreic acid, is a reported potent antibacterial that was identified more than 60 years ago, but its cellular target(s) are still unknown. Here we screen its activity against the acetyl transferase domain of a bifunctional enzyme, *Escherichia coli N*-acetylglucosamine-1-phosphate-uridyltransferase/ glucosamine-1-phosphate-acetyltransferase (GlmU). Terreic acid was found to inhibit the acetyltransferase domain of *E. coli* GlmU with an IC50 of 44.24 ± 1.85 µM.

6.4 Isolation and screening of amidase producing microorganisms

Amidases (E.C 3.5.1.4), also known as amidohydrolases, belongs to the nitrilase superfamily. It catalyzes the hydrolysis of amides to free carboxylic acids and ammonia. Therefore, these enzymes are widely used for the production of industrially important organic acids such as acrylic acid, nicotinic acid etc. These enzymes usually exhibit wide range of substrate specificity whereas some are specific for aliphatic amides, others cleave amides of aromatic acids or aryl or aryloxypropionamide. Some amidases are also able to hydrolyze amides of α- or ω-amino acids and aromatic amides and has also been reported for the production of optically active carboxylic acids.

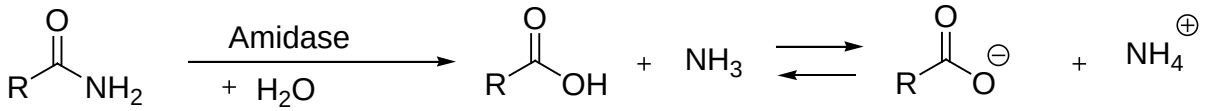


Figure 6.4.1: Amidase catalyzed biotranformation of amide to its corresponding carboxylic acid

Apart from amide hydrolysing activity, amidases also exhibit acyl-transferase activity for the hydroxamic acid production. This activity has found to involve a 'bi-bi-ping-pong' mechanism. In this mechanism, one substrate A (acyl donor) reacts with the enzyme to give an acyl-enzyme complex E *, which then transfers the acyl group to the second substrate B (acyl acceptor) which forms hydroxamic acids (Figure 6.4.2).

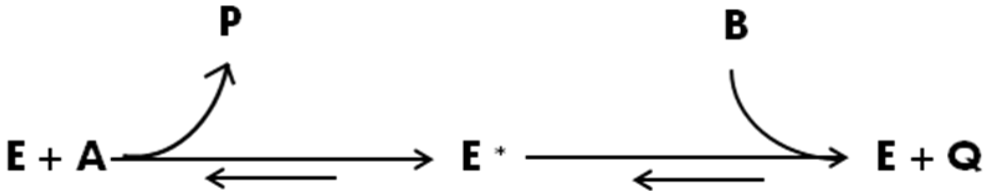


Figure 6.4.2 Enzyme-substituted mechanism. For example, A = amide, B = hydroxylamine, P = ammonia, and Q = hydroxamic acid. E = enzyme and E * = acyl-enzyme complex

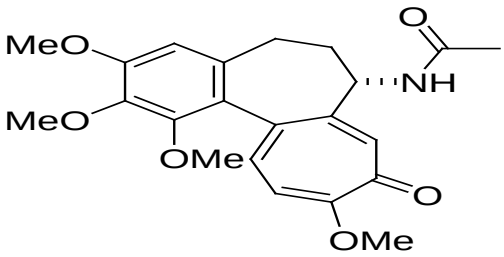
Hydroxamic acids such as acetohydroxamic acid, pyridine hydroxamate, benzohydroxamic acid etc have been synthesized by using acyltransferase activity of amidase. These compounds have been extensively investigated as an inhibitor of many enzymes such as urease, peroxidase and several matrix metalloproteases. For bio-medical applications, some hydroxamic acids have been reported for the treatment of cancer by inhibiting histone deacetylases. An amidase producing culture has been isolated from salt fields of Gujarat using propionitrile as a nitrogen source. Partial sequence of 16S rDNA has been used for the phylogenetic analysis. Sequence homology of this culture exhibits maximum homology with other reported *Brevibacterium* strains Therefore, it has named as *Brevibacterium sp.*IIIM2706. The results indicate that maximum amidase activity was shown with Isobutyramide followed by propionamide and butyramide and valeramide. No activity was observed with aromatic amides and di-amides. From these results, it can be concluded that amidase from the isolated strain has specificity for 3-4 carbon chain aliphatic amides.

7.0 ANTI-CANCER THERAPEUTICS

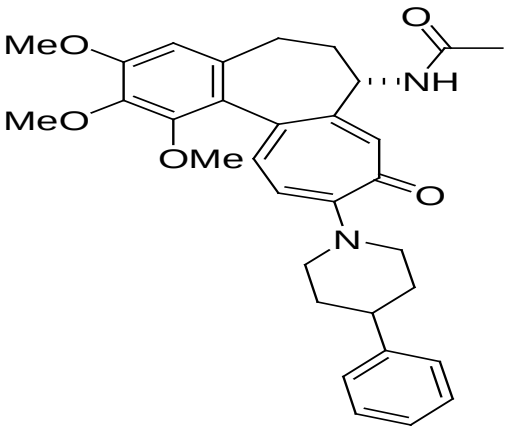
7.1 A novel microtubule depolymerizing colchicine analogue triggers apoptosis and autophagy in HCT-116 colon cancer cells

Ashok Kumar,Parduman R. Sharmaand DilipM. Mondhe

Colchicine, the main alkaloid of the plant *Colchicum autumnale*is used for the treatment of gout and familial Mediterranean fever. It is not used clinically to treat cancer due to toxicity, it does exert anti-proliferative effects through the inhibition of microtubule formation leading to mitotic arrest and cell death by apoptosis. Colchicine can still be used as a lead compound for the generation of potential anticancer drugs. Here we report the *in vitro* anticancer activity of C-ring amino-linked colchicine analogue, a semi-synthetic derivative of colchicine; N-[(7S)-1,2,3-trimethoxy-9-oxo-10-(4-phenyl-piperidin-1-yl)-5,6,7,9-tetrahydrobenzo[a]heptalen-7-yl]acetamide **(4h)** on colon cancer HCT-116 cell line.



Colchicine



Colchicine derivative **4h**

Antiproliferative effect of colchicine and **4h** was evaluated against different human cancer cell lines by MTT assay. **4h** treatment showed concentration dependent inhibition in the proliferation of HCT-116, Colo-205, A549, PC-3 and THP-1 human cancer cell lines and the respective IC_{50} values after 48 h were 1, 0.8, 1.8, 1.5 and 1.2 µM. The paclitaxel was used as positive control (Table 1). The antiproliferative activity of **4h** was evaluated against normal fR2 epithelial and normal HEK293 human embryonic kidney cells to estimate its selectivity for cancer cells for 24, 48 and 72 h treatment (Figure 7.1.1A, 1B and 1C). Compound **4h** showed better selectivity than colchicine for HCT-116 colon carcinoma cells as compared to fR2 and HEK293 cells (Figure 1D).The cell cycle distribution of **4h**-treated cells was analyzed using flow-cytometry. Cells were exposed to **4h** for 24 h before processing and analysis. As shown in Figure 7.1.2A and 2B exposure to **4h** increased the number of G2/M phase cells, while simultaneously reducing the number of cells in the S and G1 phases. This suggests a concentration-dependent induced accumulation of cells inG2/M phase and may imply that HCT-116 cells underwent cell cycle arrest.To determine if **4h** arrests cells in G2 or M phase of the cell cycle, we calculated the mitotic index of control and **4h** treated HCT-116 cells. Cells were treated with **4h** for 24 h and mitotic index was calculated (Figure 7.1.2C). In controls, 3% of the cells were in mitosis whereas, treatment with 0.75, 1.5 and 3µM **4h** resulted in concentration

dependent increase of cells with mitotic profiles (Figure 7.1.2D). Thus, **4h** was found to induce cell cycle arrest in a manner consistent with other anti-mitotic agents. Our results suggested that **4h**arrests cells in mitosis, may target microtubules in HCT-116 cells. To test this directly, wetreated cells with varying concentrations of **4h** for 24 h and analysed microtubules by immunofluorescencemicrosc opy. Control cells showed a typical array of radial,interphase microtubules (Figure 7.1.2E). A significant reduction in microtubule density was observed in concentration dependent manner. These results indicate that **4h**like colchicine depolymerizes microtubules in HCT-116 cells.Reactive oxygen species plays a prodigious role inregulating cell proliferation and removal of unwantedcells through inducing programmed cell death. The normal human cells possess certainlevel of ROS activity, but the cancer cells contain ahigher level of ROS activity due to its higher metabolicrates. If the ROS level could be elevated to a furtherextent to the threshold level, the cells push themselvesto programmed cell death. This couldbe a way to control the immortality of cancer cells.We were interested to probe if ROS would be generated in HCT-116 cells upon **4h** treatment. HCT-116 cells were loaded with the ROS probe, 2′,7′-dichlorofluorescein diacetate and H_2O_2 was included as a positive control. **4h**induced ROS generation in a concentration dependent manner, as reflected by the increase in fluorescence intensity, measured by flow-cytometry (Figure 3A and 3B) and fluorescence microscopy (Figure 7.1.3C). The highest amount of ROS was generated after exposure to 3μM of **4h**. To check whether **4h**-mediated cell death inductionwas ROS dependent, ROS accumulation study aswell as cell viability study in the presence or absenceof ascorbic acid (ROS scavenger) was performed. Inthe presence of ascorbic acid, **4h** failed to elevate ROS level (Figure 7.1.3A, 3B and 3C) and also failed to induce cell death significantly (Figure 7.1.3D), indicating thereby celldeath was ROS-mediated. In the presence of ascorbicacid (ROS scavenger), the cell growth inhibitiondecreased to 15% upon administration of 3μMconcentration of **4h**.Staining the **4h**treated HCT-116- cells with acridine orange (AO), suggested an increase of the lysosomal compartments.

Detectionandquantification of AVOs by flow-cytometry and fluorescence microscopy in**4h**-treated HCT-116 cells showed a concentration-dependent increase of AVOs (Figure 7.1.4A, 4B and 4C). To visualize the lysosomal compartments in **4h** treated cells, we stained lysosomes using lysosome associated membrane protein (LAMP)-1 directed antibody and used DAPI for nuclear staining. These staining revealed an increase in number and size of lysosomes around the nucleus of HCT-116 cells following **4h**-treatment (Figure 7.1.4D). Hence, the observed massive increase of the lysosomal compartments in HCT-116 cells as a result of **4h** treatment, suggests the stimulation of a catabolic process dependent on lysosomal degradation.**4h** treatment of HCT-116 cells results in induction of autophagy.**4h**induced autophagy wasdemonstrated by acridine orange staining of HCT-116 cells, which measures autophagic acidic vesicular organelles. We also detected changes inautophagic activity by observing the fluorescence of MDC, whichhas been known as a specific marker for autophagic vacuoles (Figure 7.1.5A and 5B). The numberof autophagic vacuoles stained by MDC in the**4h** treated HCT-116 cells was much higher than in the control. Next, to further confirm the induction of autophagy by **4h**, a set of autophagy- related factors including LC3-I and LC3-II and autophagosomes and lysosomes fusion in the HCT-116 cells after treatment with different concentrations of **4h** for 24 h were investigated by immunofluorecence

microscopy and western blot analysis (Figure 7.1.5C, 5E and 5D). Notably, the conversion of LC3-I to LC3-II, an established indicator of autophagy, was greatly enhanced by**4h**, but the prior treatment with 3-MA decreased the conversion of LC3-I to LC3-II. Autophagy can lead to either cell survival or cell death.The autophagy inhibitor, 3-MA was further used to investigate whether **4h**-induced cell death is attributed to autophagy. HCT-116 cells were treated with 3 mM 3-MA for 2 h prior to 24 h of **4h** treatment (1.5 and 3 μM). 3-MA pretreatment greatly abrogated **4h**-induced cell death (Figure 7.1.5F), indicating that autophagy contributed to the cell death in the HCT-116 cells.The final stage of autophagy is the fusion of autophagosomes with lysosomes. To examine this step, we performeddouble immunostaining with anti–LC3 and anti–LAMP-1 antibodies. HCT-116 cells were treated with 3μM of**4h**for 24h, which induced normal autophagy, including fusion between autophagosomes and lysosomes. In BEZ235 treated cells, in which autophagy was expected to be functional, LC3 and lysosomal signals significantly overlapped. In contrast, HCT-116 cells treated with bafilomycinA exhibited almost complete separation between the two signals, indicating that autophagosome-lysosome fusion was severely inhibited (Figure 7.1.6). The flow cytometric analysis with dual stain (FITCtaggedAnnexinV) revealed a progressive increment of apoptotic cell population with treatment with **4h**only as compared with control suggesting therebyinduction of apoptosis. The percentages of apoptotic cells were shown in Figure 7A. **4h**-induced apoptosis was independent of autophagy as blockage of autophagy by 3-MA failed to stop apoptosis induction (Figure 7.1.7A). HCT-116 cells showed microvilli on the cell surface while treatment with **4h**for 24 h caused concentration dependent condensation, smoothening of cell surface, loss of microvilli,blebbing of plasma membrane and the formation of apoptotic bodies in cells (Figure 7.1.7B and 7C).Colony formation ability of HCT-116 cells was attenuated by **4h**treatment significantlyin a concentration dependent manner (Figure 7.1.7D and 7E).In untreated HCT-116 cells overlapping colonies were observed.In conclusion, the microtubule depolymerizing agent **4h** with better selectivity could be used as a potential therapeutic agent in the cancer therapies with the induction of dual cell-death machineries. The present studies shed new light on the mechanisms involved in **4h**-triggered cell death and suggests that **4h**-induced ROS-triggered autophagic and apoptotic cell deaths, and that apoptotic cell death is independent of autophagy, providing a novel way toward newer cancer drug design, thus opening a new opportunity for colchicine scaffold leading to different biological activity.

Table 7.1.1**IC_{50}**values of colchicine and its analogue**4h** against different human cancer cell lines.Data are expressed as the mean ± SD.

IC50 (μM) value of colchicine and its analog **4h** against different human cancer cell lines after 48 h

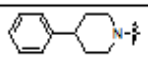
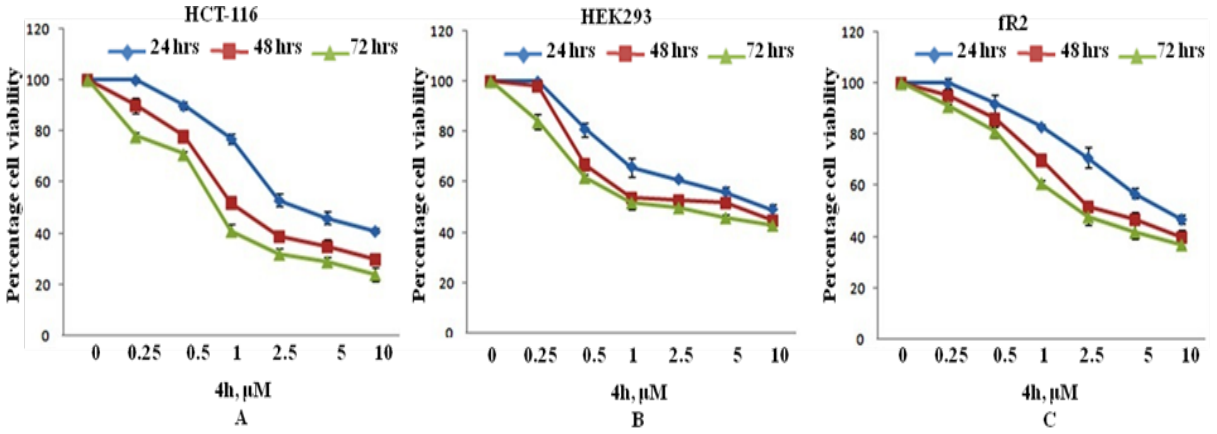
Entry	R	Colon		Lung	Prostrate	Leukemia
		HCT-116	Colo-205	A549	PC-3	THP-1
4h		1.0 ± 0.404	0.800 ± 0.15	1.8 ± 0.208	1.5 ± 0.208	1.2 ± 0.15
Colchicine	$-OCH_3$	0.05 ± 0.005	0.032 ± 0.005	0.058 ± 0.001	0.07 ± 0.009	0.01± 0.001
Paclitaxel	-	0.65± 0.055	-	0.73± 0.05	-	-
Mitomycin	-	-	-	-	0.17± 0.010	-

Table 1**IC_{50}**values of colchicine and its analogue**4h** against different human cancer cell lines.Data are expressed as the mean ± SD.



IC_{50} (µM)

Time Points	Entry	HCT-116	fr2	HEK293	Selectivity (fR2 / HCT-116)	Selectivity (HEK293 / HCT-116)
24	4h	3.5± 0.458	8.5± 0.5	9.5± 0.602	2.42	2.71
	Colchicine	0.071 ±0.001	0.073 ± 0.003	0.093 ± 0.009	1.02	1.30
48	4h	1.0 ± 0.404	3.5 ± 0.351	6.5± 0.611	3.5	6.5
	Colchicine	0.05 ± 0.001	0.05 ± 0.001	0.067± 0.003	1	1.34
72	4h	0.8± 0.05	2.3± 0.416	2.5± 0.45	2.87	3.12
	Colchicine	0.023± 0.009	0.021± 0.001	0.029± 0.007	0.91	1.26

D

Figure 7.1.1: Antiproliferative activity of **4h** against **(A)** HCT-116 colon cancer cell line, **(B)** normal human embryonic kidney cell line HEK293 and **(C)**normal breast epithelial fR2 cell line The cells were treated with various concentrations of **4h** for 24, 48 and 72 h. The levels of cell proliferation were measured by MTT assay. The percentage of viable cells was calculated as the ratio of treated cells to the control cells. The data are reported as the mean ± S.D. of three independent experiments. **(D)**Anticancer profile and selectivity index of compound **4h** and colchicine against human colon HCT-116 cell line, normal HEK293 human embryonic kidney cell line and normal epithelial fR2 cell line.

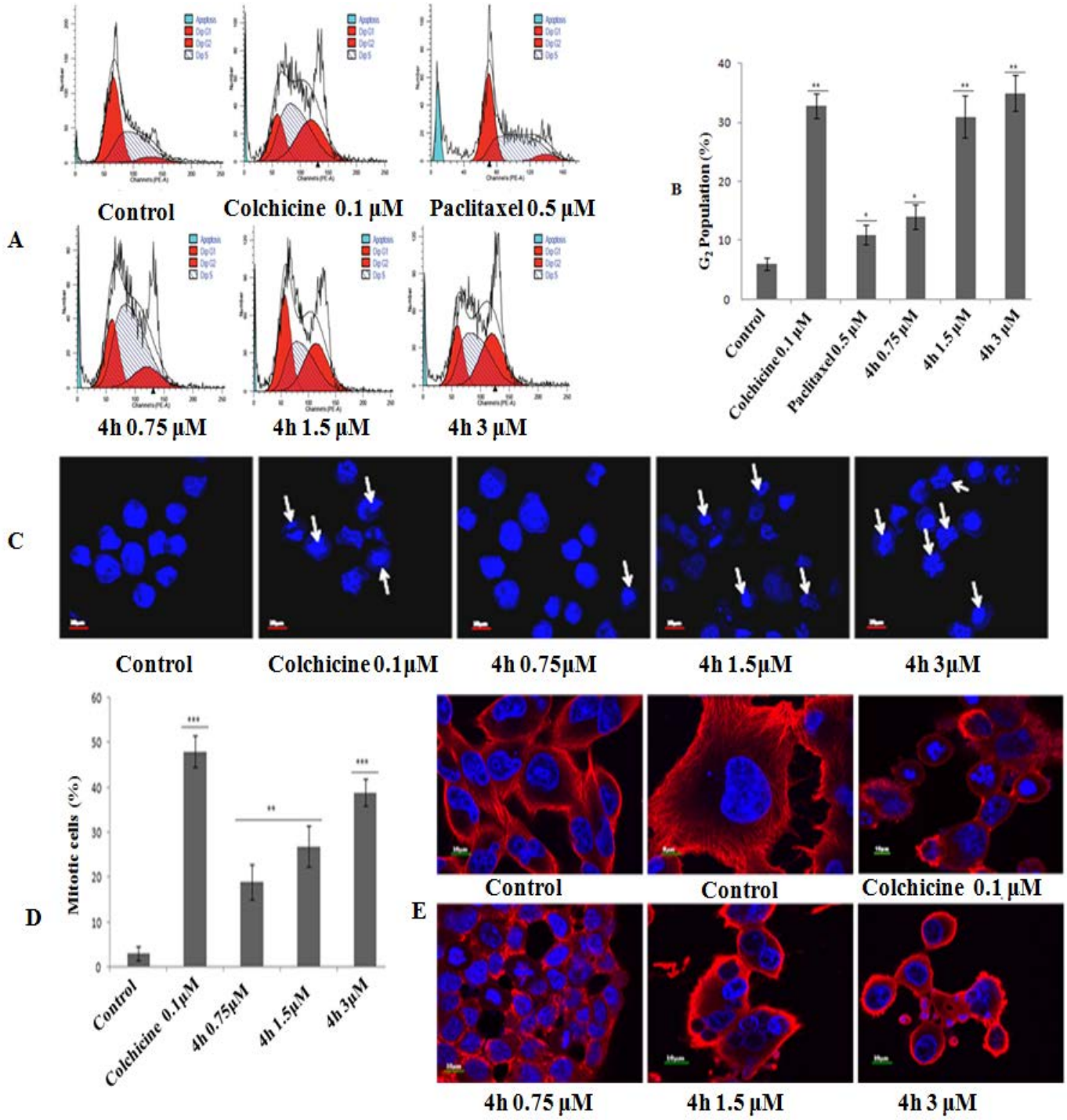


Figure 7.1.2: (A) Cell cycle analysis of **4h** treated HCT-116 cells. HCT-116 Cells (2×10^6 cells/ml/well) were exposed to indicated concentrations of **4h** for 24 h and stained with PI to determine DNA fluorescence and cell cycle phase distribution as described in materials and methods. **(B)** Data are mean ± S.D. of three similar experiments; statistical analysis was done with *P<0.05 and **P<0.01. **(C)** Effect of **4h** on mitotic index in HCT-116 cells. Cultured HCT-116 cells were treated with various concentrations of **4h** for 24 h. Cells were fixed and then stained with DAPI (1µg/ml). Mitotic index was determined by counting interphase and mitotic cells by 60X oil immersion lens using confocal microscope At least 500 cells per data point were counted. **(D)** Data are presented as mean ± S.D, of three similar experiments; Statistical analysis was done with **P<0.01 and ***P<0.001. **(E)** Compound **4h** disrupts the microtubules of HCT-116 cells. Cells were treated with indicated concentrations of colchicine and compound **4h** for 24 h.Immunocytochemical staining was conducted using anti α-tubulin antibody
and Alexa Flour-555-labeled secondary antibody and nuclei were stained with DAPI.

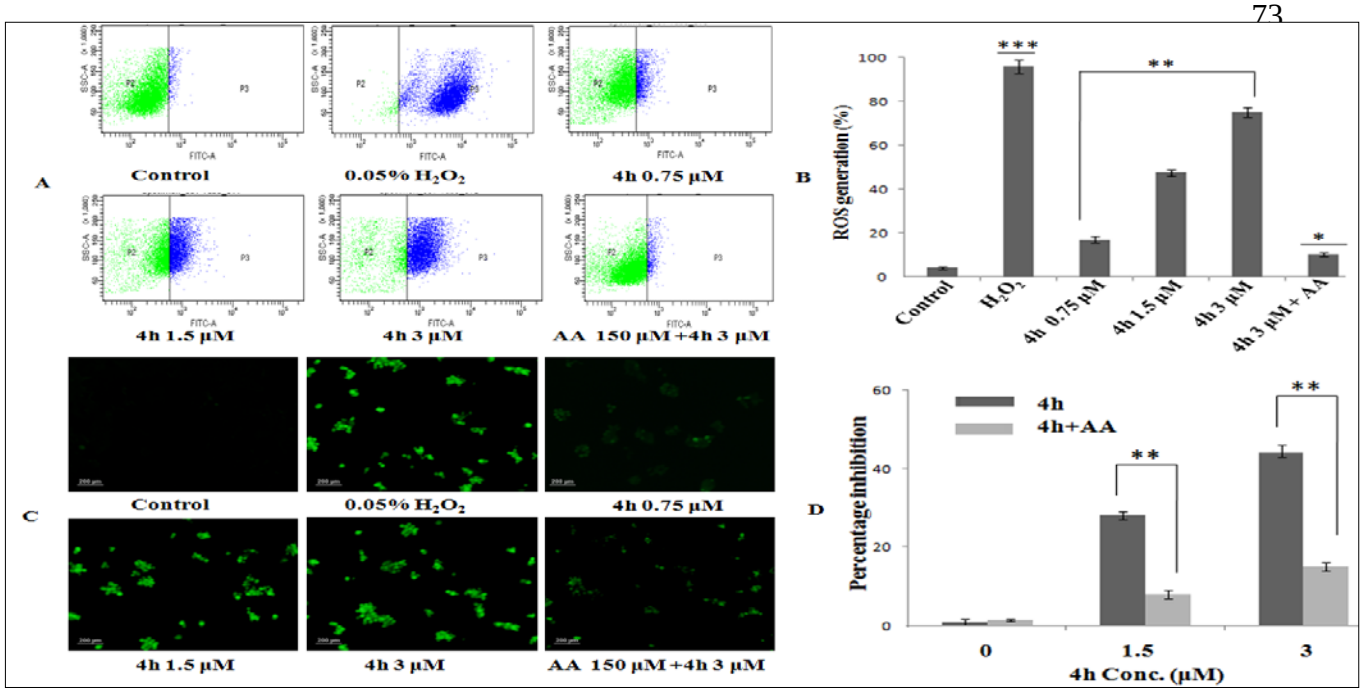


Figure 7.1.3: (**A**) Intracellular ROS level was measured by flow cytometry analysis using DCFH-DA after 24 h. Cells were treated with indicated concentrations of **4h**, 150 µM ascorbic acid and 0.05% H_2O_2 respectively and incubated with 5µM DCFH-DA. **(B)**Histogram showing percentage ROS generation by flow cytometry. Data are mean ± S.D. of three similar experiments; statistical analysis was done with p*< 0.05, p**< 0.01 and p***< 0.001.**(C)** Detection of ROS by fluorescence microscope. After incubation with DCFH-DA, cells were washed and examined by fluorescence microscope (10X). **(D)** Cell growth inhibition in the presence of ROS scavenger (Ascorbic acid). The cells were treated with **4h** (1.5 and 3 µM) in the presence or absence of AA (150 µM)). Data are mean ± S.D. of three similar experiments; statistical analysis was done with p**< 0.01.

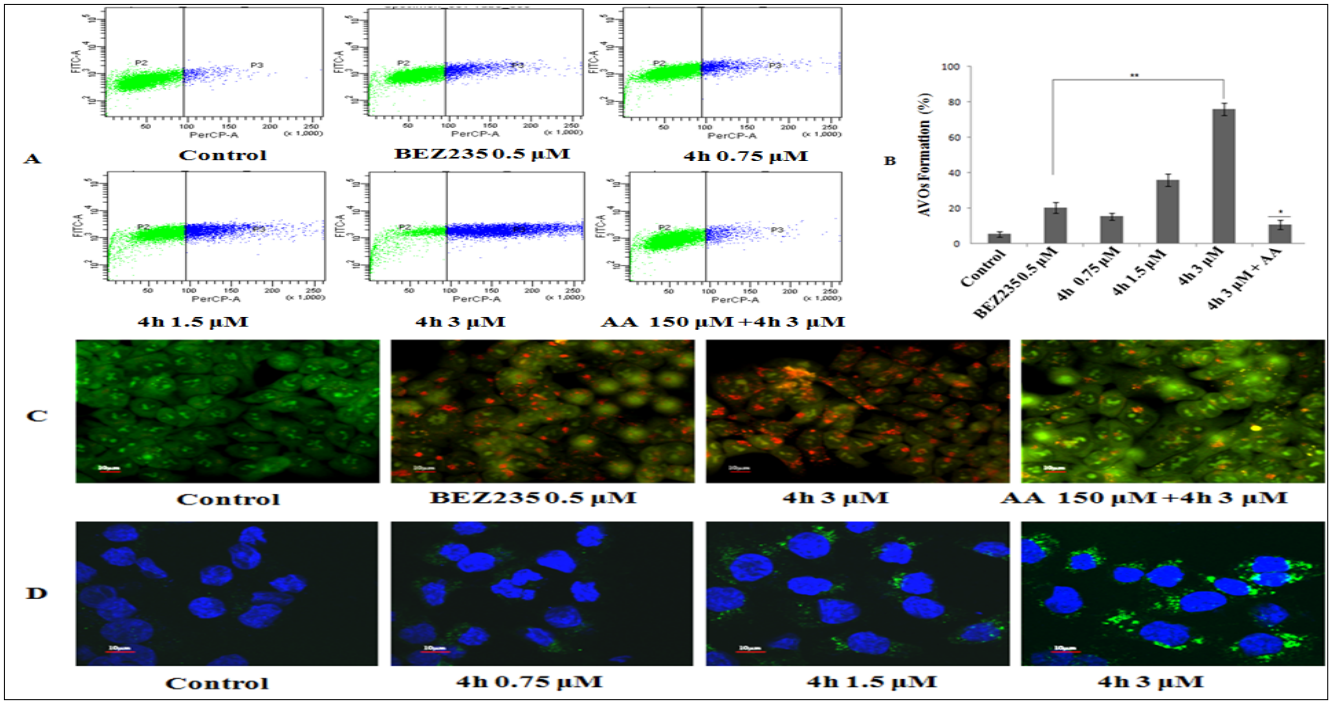


Figure 7.1.4: Increase in lyosomal compartments following **4h** treatment in HCT-116 cells. Detection and quantification of AVOs in treated HCT-116 cells. Cells were treated with indicated concentrations of compound **4h** for 24 h and stained with 1µg/ml acridine orange for 15 min, observed by **(A)** flow cytometry and **(C)** fluorescence microscopy using 40X lens. **(B)** Histogram showing percentage AVOs formation by flow cytometry. Data are mean ± S.D. of three similar experiments; statistical analysis was done with p*< 0.05 and p**< 0.01. **(D)**Immunofluorescence microscopy using LAMP-1 showed increase in size and number of perinuclear lysosomes. Nuclei were stained with DAPI.

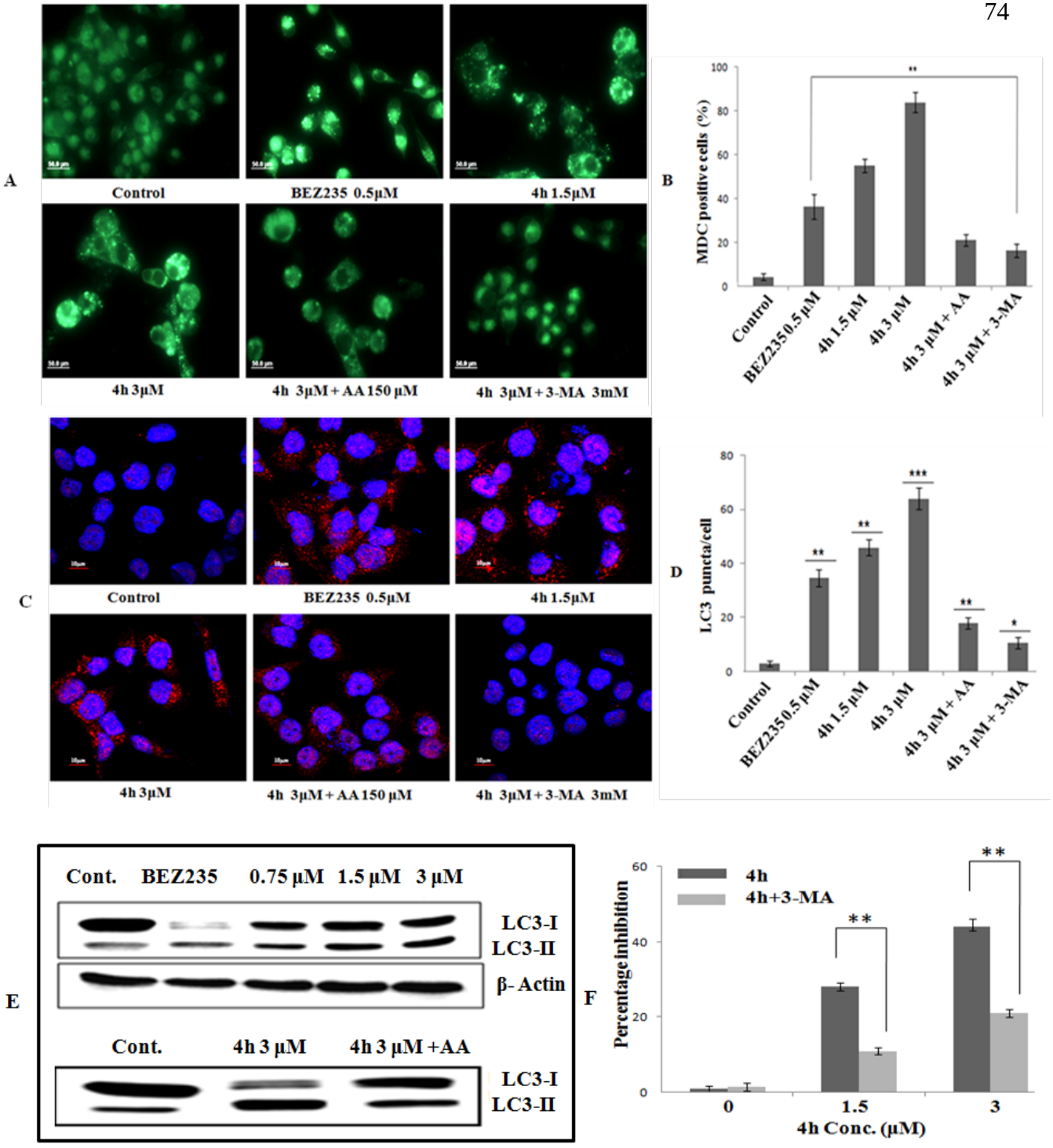


Figure 7.1.5: Compound **4h** induces autophagic cell death in HCT-116 cells. **(A)** The autophagic vacuoles were observed under fluorescence microscope (40X) with MDC staining. The treatment of compound **4h** and BEZ235 (positive control group)induced concentration dependent formation of autophagic vacuoles in HCT-116 cells after 24 h. **(C)**Detection of autophagy with LC3b antibodyusing confocal microscopy. Immunocytochemical staining was conducted using anti-LC3b antibody and Alexa Flour-555-labeled secondary antibody. Nuclei were stained with DAPI. **(D)** LC3 puncta per cell were counted and displayed in form of bar diagram. Data are mean ± S.D. of three similar experiments; statistical analysis was done with p*< 0.05, p**< 0.01 and p***< 0.001. **(E)**.Cells were lysed and the level of LC3 protein was analyzed by western blot. **(F)**Effect of pretreatment with inhibitor 3-MA on autophagic cell death by **4h** in the HCT-116 cells. Data are mean ± S.D. of three similar experiments; statistical analysis was done with p**< 0.01.

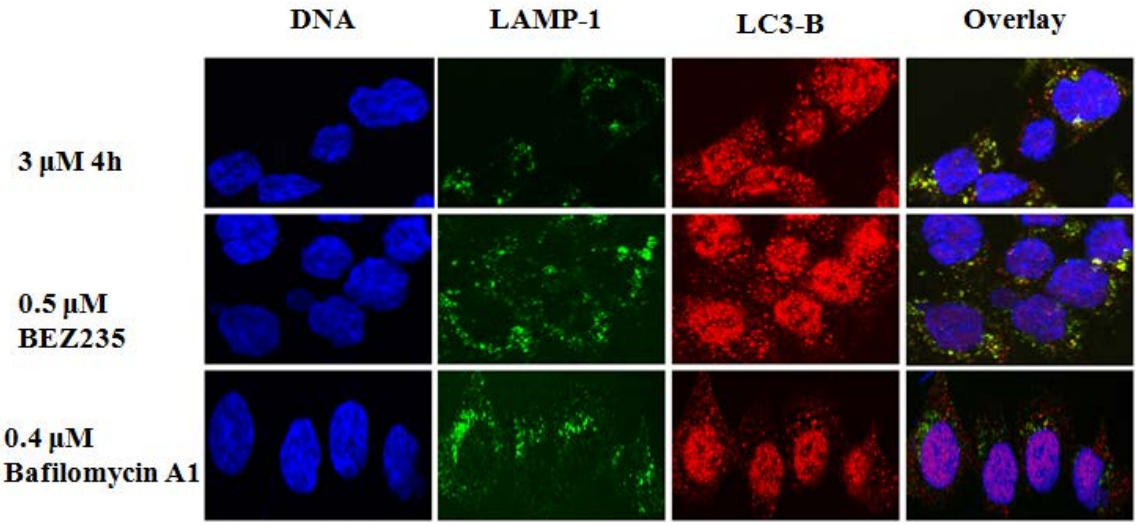


Figure 7.1.6: Double-immunocytochemical staining of HCT-116 cells with anti–LC3 and anti LAMP-1 antibodies. Cells were treated with indicated concentrations for 24 h. Fusion between autophagosomes and lysosomes (yellow) was evident in **4h** and BEZ235 treated cells respectively, but a nearly complete separation between autophagosomes and lysosomes was evident in bafilomycin A1 -treated cells.

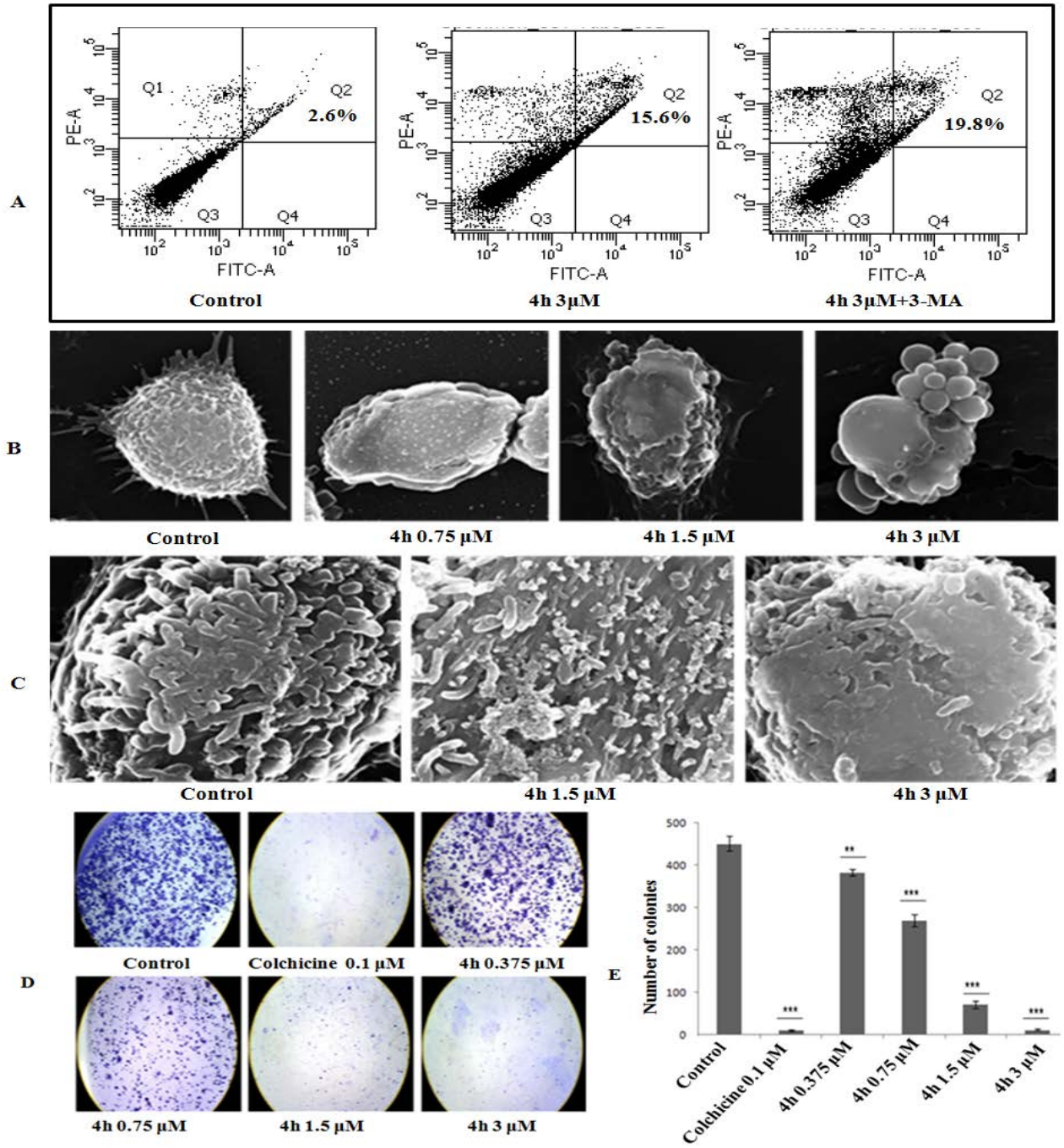
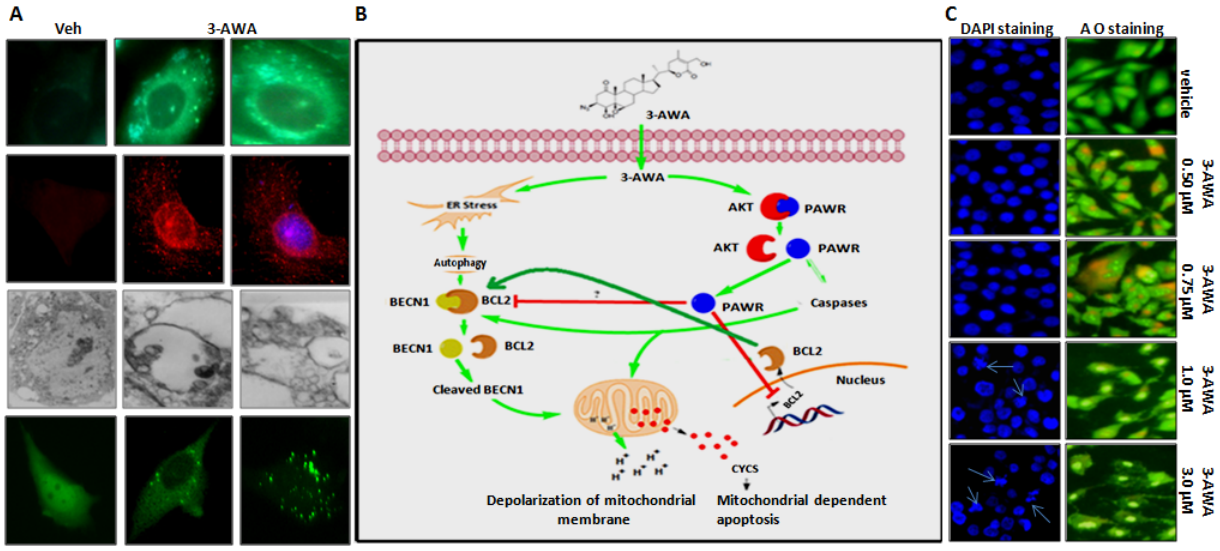


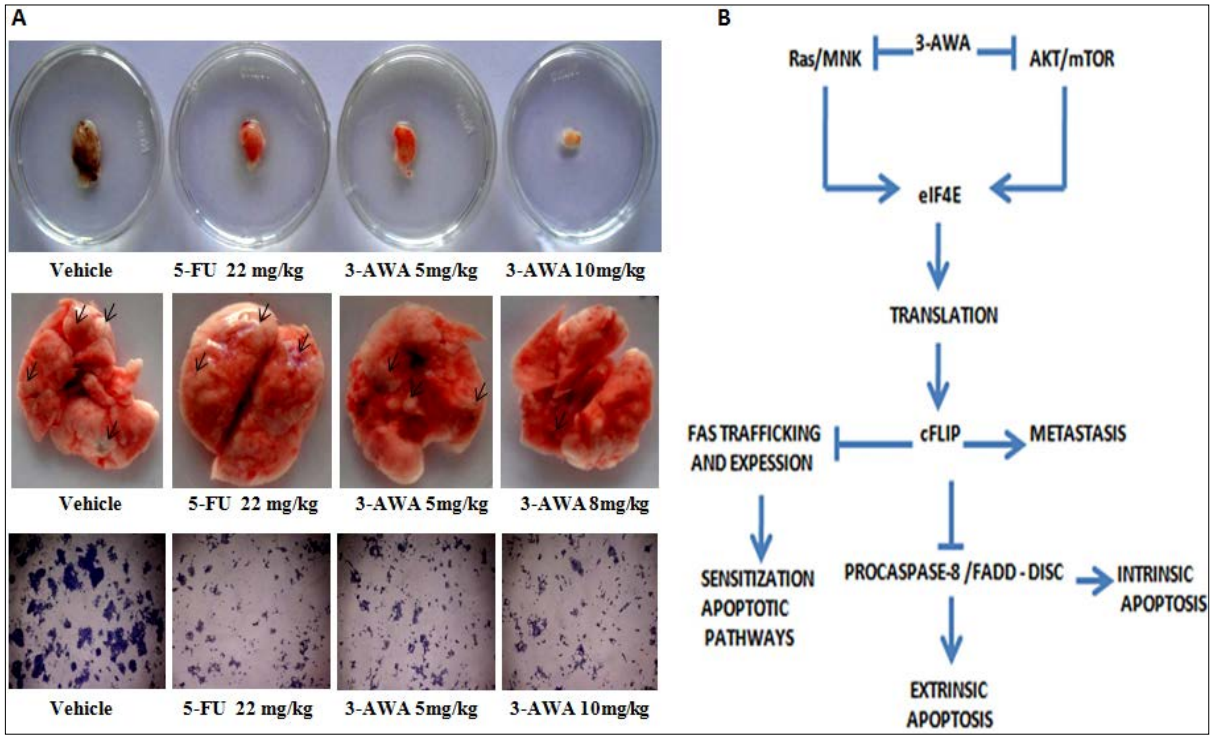
Figure 7.1.7: (A) The effects of **4h** on the exposure of phosphatidylserine by the HCT-116 cell line after 24 h treatment. Phosphatidylserine exposure was assessed by the Annexin-V / propidium iodide assay, as described in methodology and analyzed by flow cytometry.**(B, C)**Effect of compound **4h** on morphology of HCT-116 cells. SEM of untreated and treated HCT-116 cells showing surface ultrastructure. The untreated cells show microvilli on the cell surface. Treatment with **4h** after 24 h causes concentration dependent condensation, loss of microvilli and apoptosis. Magnification **(B)** 3000X and **(C)** 5000X. **(D)** Colony formation assay was carried out taking HCT-116 cells in six well plates and treated with different concentrations of **4h** for seven days. The number of crystal violet stained cells per colony was counted randomly and quantified. **(E)** Data are mean ± S.D. of three similar experiments; statistical analysis was done with **P<0.01 and ***P<0.001.

7.2 PAWR mediated BCL2 suppression promotes switching of 3-azido Withaferin A (3-AWA) induced autophagy to apoptosis in prostate cancer cells

An active medicinal component of plant origin with an ability to overcome autophagy by inducing apoptosis should be considered a therapeutically active lead pharmacophore to control malignancies. In this report, we studied the effect of concentration-dependent 3-AWA (3-azido withaferin A) sensitization to androgen-independent prostate cancer (CaP) cells which resulted in a distinct switching of 2 interrelated conserved biological processes, i.e. autophagy and apoptosis. We have observed 3 distinct parameters which are hallmarks of autophagy in our studies. First, a subtoxic concentration of 3-AWA resulted in an autophagic phenotype with an elevation of autophagy markers in prostate cancer cells. This led to a massive accumulation of MAP1LC3B and EGFP-LC3B puncta coupled with gradual degradation of SQSTM1. Second, higher toxic concentrations of 3-AWA stimulated ER stress in CaP cells to turn on apoptosis within 12 h by elevating the expression of the proapoptotic protein PAWR, which in turn suppressed the autophagy-related proteins BCL2 and BECN1. This inhibition of BECN1 in CaP cells, leading to the disruption of the BCL2-BECN1 interaction by overexpressed PAWR has not been reported so far. Third, we provide evidence that pawr-KO MEFs exhibited abundant autophagy signs even at toxic concentrations of 3-AWA underscoring the relevance of PAWR in switching of autophagy to apoptosis. Last but not least, overexpression of EGFP-LC3B and DS-Red-BECN1 revealed a delayed apoptosis turnover at a higher concentration of 3-AWA in CaP cells. In summary, this study provides evidence that 3-AWA is a strong anticancer candidate to abrogate protective autophagy. It also enhanced chemosensitivity by sensitizing prostate cancer cells to apoptosis through induction of PAWR endorsing its therapeutic potential.

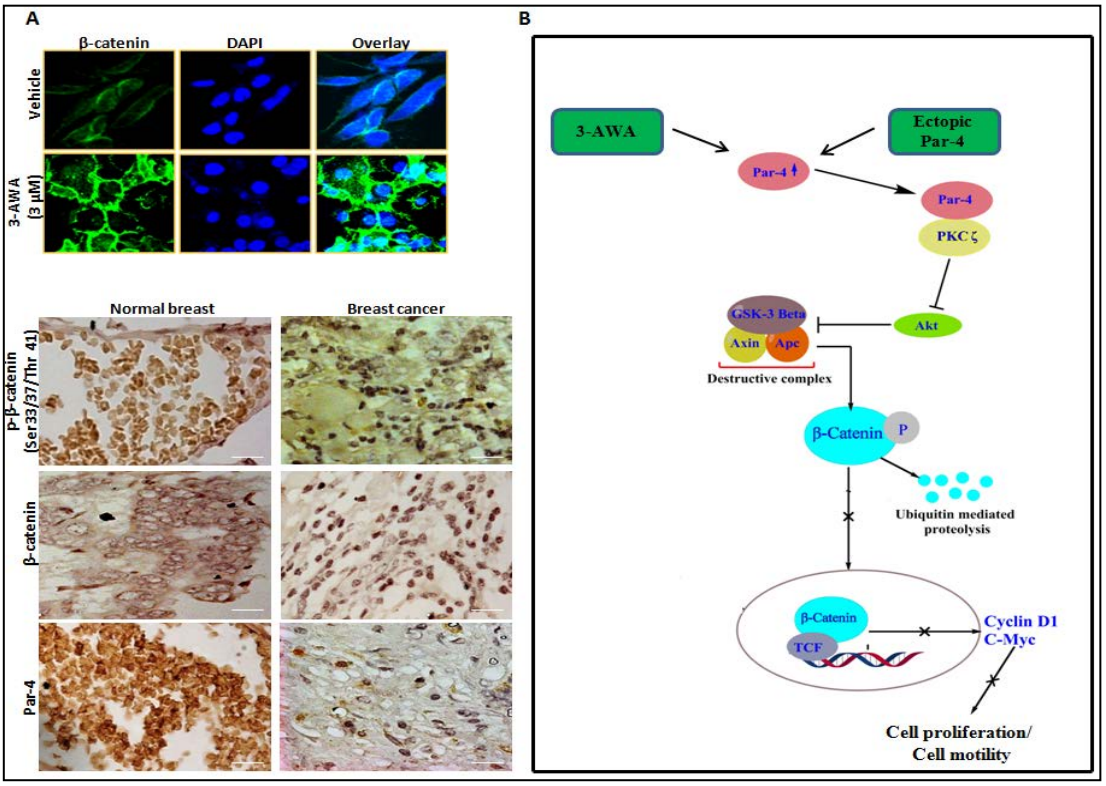


7.3 Dual modulation of Ras-Mnk and PI3K-AKT-mTOR pathways: Novel c-FLIP inhibitory mechanism of 3-AWA mediated translational attenuation through dephosphorylation of eIF4E



The eukaryotic translation initiation factor 4E (eIF4E) is considered as a key survival protein involved in cell cycle progression, transformation and apoptosis resistance. Herein, we demonstrate that medicinal plant derivative 3-AWA (from Withaferin A) suppressed the proliferation and metastasis of CaP cells through abrogation of eIF4E activation and expression via c-FLIP dependent mechanism. This translational attenuation prevents the *de novo* synthesis of major players of metastatic cascades *viz.* c-FLIP, c-Myc and cyclin D1. Moreover, the suppression of c-FLIP due to inhibition of translation initiation complex by 3-AWA enhanced FAS trafficking, BID and caspase 8 cleavage. Further ectopically restored c-Myc and GFP-HRas mediated activation of eIF4E was reduced by 3-AWA in transformed NIH3T3 cells. Detailed underlying mechanisms revealed that 3-AWA inhibited Ras-Mnk and PI3-AKTmTOR, two major pathways through which eIF4E converges upon eIF4F hub. In addition to *in vitro* studies, we confirmed that 3-AWA efficiently suppressed tumor growth and metastasis in different mouse models. Given that 3-AWA inhibits c-FLIP through abrogation of translation initiation by cotargeting mTOR and Mnk-eIF4E, it (3-AWA) can be exploited as a lead pharmacophore for promising anti-cancer therapeutic development.

7.4 Par-4 dependent modulation of cellular β-catenin by medicinal plant natural product derivative 3-azido Withaferin A.



Here, we provide evidences that natural product derivative 3-azido Withaferin A (3-AWA) abrogated EMT and invasion by modulating b-catenin localization and its transcriptional activity in the prostate as well as in breast cancer cells. This study, for the first time, reveals 3-AWA treatment consistently sequestered nuclear b-catenin and augmented its cytoplasmic pool as evidenced by reducing b-catenin transcriptional activity in these cells. Moreover, 3-AWA treatment triggered robust induction of pro-apoptotic intracellular Par-4, attenuated Akt activity and rescued Phospho-GSK3b (by Akt) to promote b-catenin destabilization. Further, our in vitro studies demonstrate that 3-AWA treatment amplified Ecadherin expression along with sharp downregulation of c-Myc and cyclin D1 proteins. Strikingly, endogenous Par-4 knock down by siRNA underscored 3-AWA mediated inhibition of nuclear b-catenin was Par-4 dependent and suppression of Par-4 activity, either by Bcl-2 or by Ras transfection, restored the nuclear b-catenin level suggesting Par-4 mediated b-catenin regulation was not promiscuous. In vivo results further demonstrated that 3-AWA was effective inhibitor of tumor growth and immunohistochemical studies indicated that increased expression of total b-catenin and decreased expression of phospho-b-catenin and Par-4 in breast cancer tissues as compared to normal breast tissue suggesting Par-4 and b-catenin proteins are mutually regulated and inversely co-related in normal as well as cancer condition. Thus, strategic regulation of intracellular Par-4 by 3-AWA in diverse cancers

could be an effective tool to control cancer cell metastasis. Conclusively, this report puts forward a novel approach of controlling deregulated b-catenin signaling by 3-AWA induced Par-4 protein.

7.5 Design and synthesis of antitumour Heck coupled Sclareol analogs: Modulation of BH3 family members by SS-12 in autophagy and apoptotic cell death.

Sclareol, a promising anticancer labdane diterpene, was isolated from Salvia sclarea. Keeping the basic stereochemistry-rich framework of the molecule intact, a method for the synthesis of novel sclareol analogues was designed using palladium (II)-catalyzed oxidative Heck coupling reaction in order to study their structure−activity relationship. Both sclareol and its derivatives showed an interesting cytotoxicity profile, with 15-(4-fluorophenyl) sclareol (SS-12) as the most potent analogue, having IC50 = 0.082 μM against PC-3 cells. It was found that SS-12 commonly interacts with Bcl-2 and Beclin 1 BH3 domain proteins and enhances autophagic flux by modulating autophagy-related proteins. Moreover, inhibition of autophagy by autophagy inhibitors protected against SS-12-induced apoptosis. Finally, SS-12 effectively suppressed tumor growth in vivo in Ehrlich's ascitic and solid Sarcoma-180 mouse models.

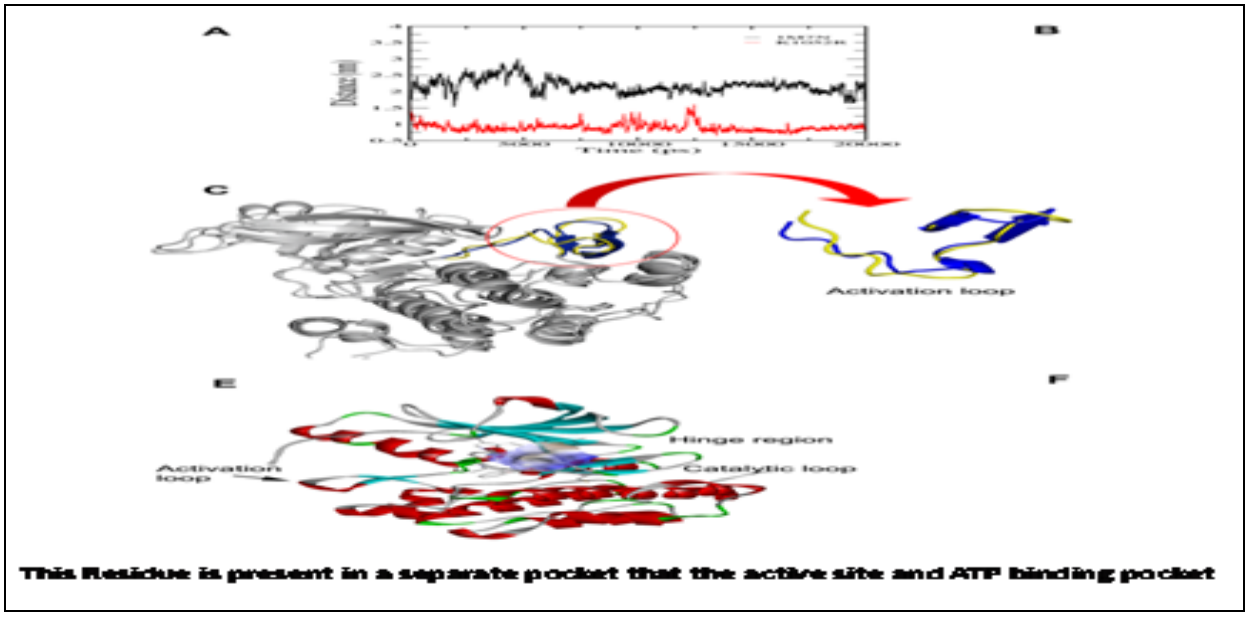
7.6 Development of IGF1R cell-based assay and identification of an allosteric site in IGF1R

The insulin-like growth factor-I receptor (IGF-1R) is a member of the receptor tyrosine kinase family. The IGF pathway is commonly dysregulated in many human cancers, including breast, prostate, liver, lung, bladder, thyroid, renal cancers, Ewing's sarcomas, rhabdomyosarcoma, lymphomas, leukemias, multiple myeloma, etc. . The insulin receptor (IR) is a related receptor tyrosine kinase and shares a high sequence identity (~84%) in the tyrosine kinase domain with IGF-1R. This homology makes it very difficult to target one of the receptors selectively and creates a special challenge for IGF-1R inhibitor design. Due to the very high degree of homology among the catalytic domains of IGF1R and IR , all of the known advanced IGF1R tyrosine kinase inhibitors (TKIs) inhibit IR to a significant degree, as well. Over the past decade, the obvious selectivity problem associated with small molecules has led to a shift in interest towards the development of an intrinsically, highly selective monoclonal antibody or protein-based IGF1R blockers which target either the receptor itself or its ligands. Thus identifying allosteric sites in a protein is a promising approach to inhibit one target and to spare the others with close homology.We have recently identified a unique binding pocket in the C-terminal domain of IGF-1R which is different than the ATP binding pocket. This pocket falls in the vicinity of activation loop and upon mutating residues in this pocket the autophosphorylation activity of IGF-1R and the subsequent down stream signaling is drastically reduced. Thus, we aim to explore small molecule inhibitors that can bind to this pocket and act as allosteric inhibitors for IGF1R. Recently, we have developed a cell based assay using HEK293 cells for screening small molecules against IGF-1R. This assay involves transfection of HEK293 cells with constructs corresponding to

IGF-1R which is then followed by the immunoblotting with phosphor-specific antibodies. This assay also helps in the downstream pathway analysis using these constructs. While developing this assay we generated many loss-of-function and gain of function mutatants of IGF1R. Upon analyzing these mutants we identified a unique mutations in IGF1R which is residing in a pocket not involved in ATP binding. Autophosphorylation activity and downstream signaling activity of this mutant, identified in the new binding pocket, showed that this site could be exploited for the development of allosteric small molecules inhibitors of IGF1R.

Figure 7.6.1 Lane pattern: Wt, M, C, DM, and TM represent the overexpression of IGF-1R proteins in HEK293 cells to assess the autophosphorylation activity and dowmstream signalling (pAkt levels). Wt-wildtype protein, M-IGF1R with single mutation, Control, DM-IGF1R with double mutation, TM-IGF1R with triple mutation.

As can be seen in Figure 7.6.1 (above) wherever this mutation was incorporated in IGF1R (whether as single , double or triple mutation) phospho-IGF1R as well as pAkt levels are reduced. Furthermore, we performed bioinformatic analysis of this binding pocket and observed that this pocket indeed is different than the substrate binding pocket and a perfect for designing allosteric inhibitors of IGF1R as shown in Figure 7.6.2 (below)



8.0 Animal House

8.1 Towards establishment of facility for early prediction of invivo efficacy of anticancer Compounds/products: Hollow fiber mouse model to facilitate invivo anticancer drug screening.

Yadav Govind, Nagar Rakesh Kumar, Choudhary Amit kumar

Several preclinical *in vitro* and *in vivo* tumor models based on human cancer cell lines are being used for discovery and evaluation of new anticancer drugs. Many *in vitro* tumor models cannot replicate the complex tumor microenvironment and pharmacokinetics of drugs *in vivo*, therefore, a demonstration of *in vivo* drug efficacy is required after the *in vitro* screening process. The xenograft mouse model is the conventional method used for preclinical *in vivo* drug testing; however, it requires significant numbers of mice and quantities of test compounds and incurs high costs in terms of both labor and time. In addition, some human cancer cell lines do not readily form tumors in animals (Sharma sv *etal* 2010 ,Philips RM *etal*1990). The hollow fiber assay is based on techniques for cultivation of mammalian cells in hollow fibers permeable to substances with a molecular weight<500,000 Da, and implantation of the fibers in various body compartments of mice, followed by drug exposure and quantitation of the viability of the cells. The hollow fiber assay is a unique *in vivo* model, which allows simultaneous evaluation of up to 6 different cell lines in 2 physiological separate compartments. The hollow fiber model has a shorter evaluation time and reduced compound requirement than traditional xenografts models(Hollingshead *etal* 1995). The model allows for the effective pairing of a novel compound, with the appropriate cell line, by its capacity to utilize multiple. ***Animals: Athyamic nude 6-8 week of age***. All animal experiments were carried out according to the "Principles of Laboratory Animal Care" and the Guidelines of the Institutional Animal Ethics Committee, Indian institute of integrative medicine (IIIM-CSIR) Jammu, India.

Cells and chemical, drug : **PC3, Ovcar-5, COLO205, HeLa, MCF7, HL60, HCT15,** cells were cultured in their standard suitable and adapted mainly in RPMI-1640 medium containing 10% fetal calf serum (FCS), 1% L-glutamine (2 mM), and 1% sodium pyruvate (1 mM) (RPMI-10) (all from Sigma-Aldrich), in a humidified incubator at 37 °C, 5% CO_2. To evaluate cytotoxicity, the standard US NCI protocol was followed, with Paclitaxel 15mg/kg, 5FU 24.5mg/kg Body weight (standard anticancer drugs) respectively and Normal saline as negative control

Hollow fiber assay To identify the in vivo anticancer activity of compound the hollow fiber assay was carried out. The hollow fiber procedure was modified from Hollingshead (1995). Modified polyvinylidene difluoride hollow fibers (Spectrum Laboratories Inc., Houston, TX) were prepared as described previously (Shnyder et al., 2006). Fibers were initially hydrated by flushing through with 70% ethanol solution followed by distilled water, and then autoclaved (121 °C for 15 min) to sterilize. The fibers were inoculated with 2.5×10^6 cells/ml densities each for cells using a 5-mL syringe with a 18 G needle. The fibers were sealed with a hot smooth-jawed needle holder at the two ends and at intervals of 2 cm. These fibers were then incubated in Petri dishes with

supplemented medium at 37℃ in a 5% CO2 for 24 h. Nude mice were anaesthetized by i/p inj of Ketamine (100mg/kg bwt) and xylasine(10mg/kg bwt). The fibers were implanted subcutaneously on day 1 into the subcutaneously to standardize propagation of cells in Hollow fiber implanted in the nude mice using a sterile trocar. To evaluate cytotoxicity, the standard US NCI protocol was followed, with Paclitaxel 15mg/kg, 5FU 24.5mg/kg Body weight (standard anticancer drugs) respectively and Normal saline as negative control administered i.p. daily for four days (3–7 days) following implantation, hollow fibers were seeded with 2.5×10^6 cells/mL of each cell line, taking into account assay time of 7 days. Each mouse was implanted with 3 color coded hollow fibers at the s.c. sites, each containing a different cell line. At the end of the assay on day 7 fibers were removed for analysis using a modified MTT assay (Hollingshead et al., 1995). Briefly, explanted fibers were wiped to remove any host tissue outside the fibers and placed into 6-well plates containing 2 mL of pre-warmed RPMI-1640/20%FCS. A 1 in 5 dilution of stock MTT solution (5 mg/mL 3-(4,-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide in water) was prepared in the same medium and 1 mL added to each well before placing in a humidified incubator at 37 °C, 5% CO_2 for 4 h. The MTT solution was aspirated and 2 mL of 2.5% protamine sulfate added to each well and the plate stored at 4 °C, shielded from the light for a minimum of 24 h. The protamine sulfate was then aspirated and replaced with 2 mL of fresh protamine sulfate and stored at 4 °C for a minimum of 2–4 h. fibers were removed, cut in half, and placed into individual wells of 24-well plates and left overnight in a safety cabinet with the light off and the airflow on to dry them. Once dried, 250 µL of DMSO was added to each well, and the plate placed on a shaker for 4 h shielded from the light to solubilize the formazan. 150 µL of the resultant solution was transferred to a 96-well plate and the absorbance at 540 nm measured using a microplate spectrophotometer. A 50% or greater reduction in percent net growth in the treated samples compared to the vehicle control samples is considered a positive result.

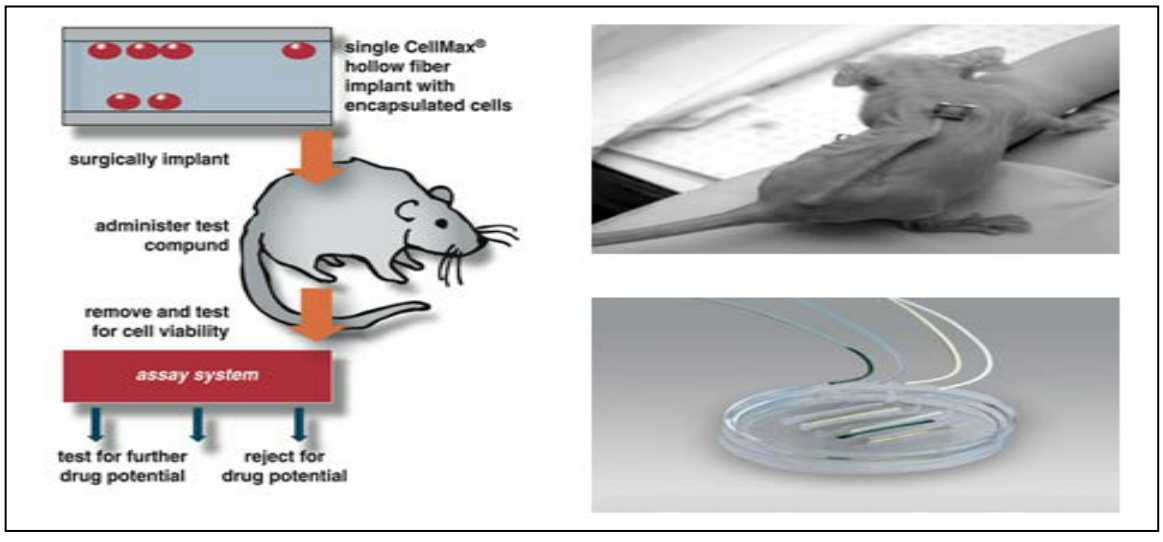
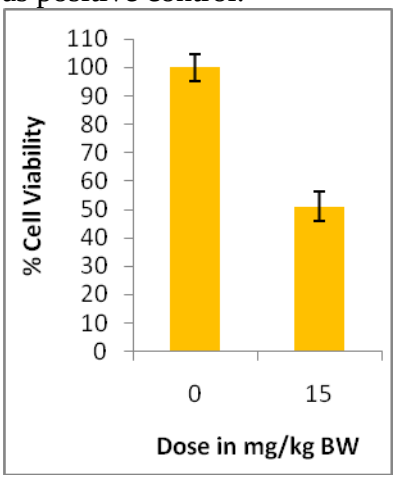
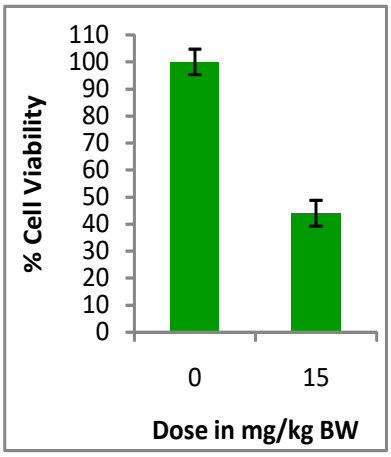


Figure 8.1.1: Showing methodology and hollow fibers (Spectrum lab)

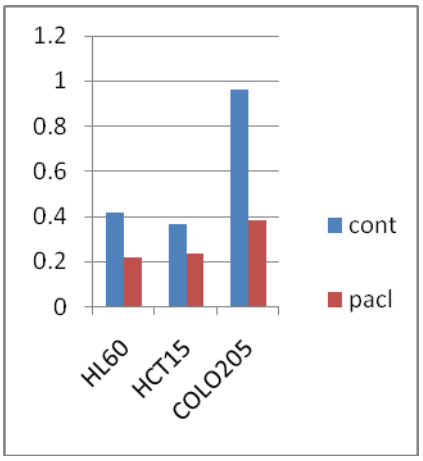
Standardization in vivo hollow fiber mouse model (for prostate, ovarian, Breast, colorectal, cervix, Leukemia cancer) using Paclitaxel and 5FU as per their sensitivity (conducted elsewhere) as positive control.



PC3

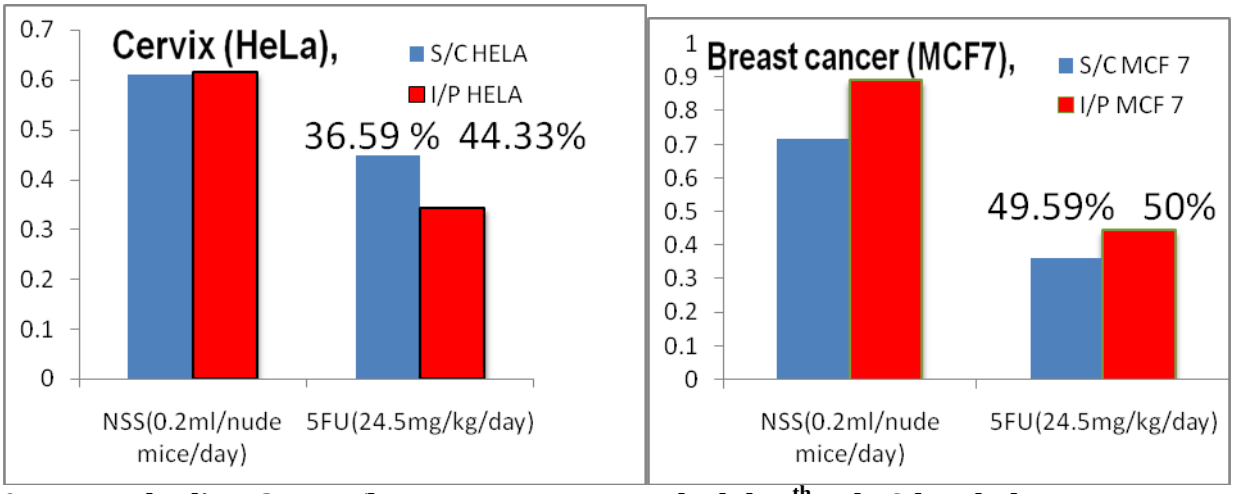


OVACAR-5



HL60,HCT15,COLO205

Figure 8.1.2: Normal saline; 15- Paclitaxel 15mg/kg BW; Treatment schedule 4th, 5th, 6th, 7th days



0- Normal saline; 24.5mg/kg- 5FU; Treatment schedule 4th, 5th, 6th, 7th days;

Assay	HeLa	MCF7	Published Ref:
Invivo Hollow fiber assay	**40**	**77**	***Lee etal 2005 Cancer Res Treat. Correlation of Hollow fiber and Xenograft Assay***
Xenograft model 8 days treatment with 24.5mg/kg 5FU	**46**	**89**	***Lee etal 2005***

8.1.1 Facility available:

Table: Available NCI panel of 12 Cells of 6 human tumors

S.No.	Selected tumor type for NCI ‘ s In Vivo hollow fiber		
1	Colon	COLO-205	SW-620
2	BREAST	MDA-MB-231	MDA-MB-435
3	LUNG	NCI-H522	NCI-H23
4	OVARIAN	OVCAR-5	OVCAR-3
5	CNS	U251	SF-295
6	MELANOMA	UACC -62	LOX IMVI

Facility will be available for Athymic Nude mice to perform assay, production and experimentation in Clean Air supplied (HEPA Filtered) animal rooms.

Figure 8.1.3: upcoming GLP Standard Invivo Laboratory(Animal house Facility) at IIIM,Jammu

Growth condition standardized using cancer cells frequently being utilized by various research groups for screening of new anticancer compounds found satisfactorily compared to earlier 8 days treatment studies (*Lee etal 2005*). Further, infrastructure created and available for conducted screening anticancer compounds using NCI 12 Cell panel of 6 different tumors and new GLP Standard Invivo Laboratory (Animal house facility) will provide immunodifficient mice and Clean room housing will support the facility for early prediction of invivo efficacy of anticancer Compounds/products.

8.1.2 Mutagenicity testing facility

Following compounds(facility code) were evaluated for mutagenicity with and without S9 Meatabolic activation system.

S.No.	Facility code Of the compound received for testing	**Strain used** *(OECD471)* in Bacterial reverse mutation test ***Salmonella/E.coli strains***						Maximum Dose/plate (µg)	**Results**
		TA98	TA1537	TA100	TA1535	TA102	Ecoliwp2uvrA		
1	**AMS-11**	-	+	-	-	-	-	5000-0.00256	**Mutagenic**
2	**AMS-12**							5000-0.00256	**Mutagenic**
3	AMS-13	-	-	-	-	-	-	5000-0.00256	Non mutagenic
4	AMS-14	-	-	-	-	-	-	5000-0.00256	Non mutagenic
5	AMS-16	-	-	-	-	-	+	5000-0.00256	**Mutagenic**
6	AMS-17	-	ND	-	ND	ND	ND	5000-0.00256	**Non mutagenic**
7	AMS-18	-	ND	-	-	ND	-	5000-0.00256	**Non mutagenic**
8	AMS-19	-	ND	-	-	ND	-	5000-0.00256	**Non mutagenic**

- Non mutagenic, + Mutagenic, ND Not done

8.1.3 Revenue Generated

IIIM animal facility supporting Research and Development of number of research Institutions by providing Research animals on request, merit and importance of the project and necessary approvals.

Year	**No. of R&D Institution supported**	**No. of Invivo study**	**No. of Research Lab Animals contributed in R&D of other institutes**	**Revenue generated (Rs)**
2015-16	**07**	**21**	**1533**	**267900**

PUBLICATIONS 2015

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170. Akindele Abidemi James; Wani Zahoor; Mahajan Girish; Sharma Sadhana; Mondhe Dilip Manikrao; Aigbe Flora Ruth; Adeyemi Olufunmilayo Olaide; Satti Naresh. Anticancer activity of Aristolochia ringens Vahl. (Aristolochiaceae). *Journal of traditional and complementary medicine* **(2015),** 5(1), 35-41. IF= Not Yet

171. Sen A; Sanyal U; Goswami K K; Mallick A; Baral R; Saxena A K. In vitro and in vivo evaluation of 2-chloroethylnitrosourea derivatives as antitumor agents. *Experimental oncology* **(2015)**, 37(1), 23-29. IF= Not Yet.

LIST OF PATENTS (2015-2016)

A. Patents filed in India

SNo	Title	Inventors	NFNO	Application No. & Date	Remarks
1	FUSED PYRIMIDINES AS ISOFORM SELECTIVE PHOSPHOINOSITIDE-3-KINASE-ALPHA INHIBITORS AND PROCESS FOR PREPARATION THEREOF	BHARATE SANDIP BIBISHAN, BHUSHAN SHASHI, MOHAMMED SHABBER, GURU SANTOSH KUMAR, BHARATE SONALI SANDIP, KUMAR VIKAS, MAHAJAN GIRISH, MINTOO MUBASHIR JAVED, MONDHE DILIP MANIKRAO, VISHWAKARMA RAM	0222NF2015/IN	3818DEL2015 & date 23-11-2015	---
2	3-PYRIMIDINYL PYRROLO [2,3-b] PYRIDINE AS NEW ANTICANCER AGENTS AND THE PROCESS FOR THE PREPARATION THEREOF	UMED SINGH, GOUSIA CHASHOO, GIRISH MAHAJAN, THANUSHA THATIKONDA, PRIYA MAHAJAN, HARI PRASAD ARURI, SATISH SONBARAO GUDUP, AMIT NARGOTRA, DILIP MANIKRAO MONDHE, RAM ASREY VISHWAKARMA, PARVINDER PAL SINGH	0211NF2015/IN	3893DEL2015 & date 30-11-2015	---
3	STANDARDIZED EXTRACT OF BERGENIA CILIATA FOR THE TREATMENT OF INFLAMMATORY DISORDERS	Bharate Sandip Bibishan, Singh Surjeet, Singh Gurdarshan, Jain Shreyans Kumar, Kumar Ajay, Singh Bikarma, Gupta Ajai Prakash, Anand Rajneesh, Singh Amarinder, Kushwaha Manoj, Gupta Mehak, Vishwakarma Ram, Sharma Ashwani, Sharma Gourav	0163NF2015/IN	201611002826 & date 27-01-2016	---
4	SUBSTITUTED AURONE ALKALOIDS AS ANTI-MYCOBACTERIAL AGENTS	Satish Sonbarao Gudup, Sanjay Kumar, Hari Prasad Aruri, Umed Singh, Gurunadham Munagala, Kushalava Reddy Yempalla, Samsher Singh, Inshad Ali Khan, Vishwakarma Ram Asrey, Parvinder Pal Singh	0169NF2015/IN	201611007477 & date 03-03-2016	---

B. Patents filed in Abroad

S.No	Title	Inventors	NFNO	Application No.	Remarks
1	POLYALKYLATED ACYL AND BENZOYL-PHLOROGLUCINOLS AS POTENT P-	BHARATE SANDIP, KUMAR AJAY, BHARATE JAIDEEP, JOSHI	0060NF2014/WO	PCT/IN2015/050069 & date 20-07-2015	

	GLYCOPROTEIN INDUCERS	PRASHANT, WANI ABUBAKAR, MUDUDUDDLA RAMESH, SHARMA ROHIT, VISHWAKARMA RAM			
2	ALKYLIDENE PHOSPHONATE ESTERS AS P-GLYCOPROTEIN INDUCERS	BHARATE SANDIP, KUMAR AJAY, MANDA SUDHAKAR, JOSHI PRASHANT, BHARATE SONALI, WANI ABUBAKAR, SHARMA SADHANA, VISHWAKARMA RAM	0058NF2014/WO	PCT/IN2015/050110 & date 16-09-2015	
3	SUBSTITUTED 1,2,3-TRIAZOL-1-YL-METHYL-2,3-DIHYDRO-2-METHYL-6-NITROIMIDAZO[2,1-b]OXAZOLES AS ANTI-MYCOBACTERIAL AGENTS AND A PROCESS FOR THE PREPARATION THEREOF	YEMPALLA KUSHALAVA REDDY, MUNAGALA GURUNADHAM, SINGH SAMSHER, SHARMA SUMIT, KHAN INSHAD ALI, VISHWAKARMA RAM ASREY, SINGH PARVINDER PAL	0176NF2014/WO	PCT/IN2015/050111 & date 16-09-2015	
4	NEW CHROMONE ALKALOID DYSOLINE FOR THE TREATMENT OF CANCER AND INFLAMMATORY DISORDERS	VISHWAKARMA RAM ASREY, JAIN SHREYANS KUMAR, BHARATE SANDIP BIBISHAN, DAR ABID HAMID, KHAJURIA ANAMIKA, MEENA SAMDARSHI, BHOLA SUNIL KUMAR, QAZI ASIF KHURDHID, HUSSAIN AASHIQ, SIDIQ TABASUM,	0037NF2013/CA	2,909,280 & date 09-10-2015	

		UMA SHAANKER RAMANAN, RAVIKANTH GUDASALAMANI, VASUDEVA RAMESH, MOHANA KUMARA PATEL, GANESHAIAH KOTIGANAHALLI			
5	NEW CHROMONE ALKALOID DYSOLINE FOR THE TREATMENT OF CANCER AND INFLAMMATORY DISORDERS	VISHWAKARMA RAM ASREY, JAIN SHREYANS KUMAR, BHARATE SANDIP BIBISHAN, DAR ABID HAMID, KHAJURIA ANAMIKA, MEENA SAMDARSHI, BHOLA SUNIL KUMAR, QAZI ASIF KHURDHID, HUSSAIN AASHIQ, SIDIQ TABASUM, UMA SHAANKER RAMANAN, RAVIKANTH GUDASALAMANI, VASUDEVA RAMESH, MOHANA KUMARA PATEL, GANESHAIAH KOTIGANAHALLI	0037NF2013/US	14/783878 & date 12-10-2015	
6	NEW CHROMONE ALKALOID DYSOLINE FOR THE TREATMENT OF CANCER AND INFLAMMATORY DISORDERS	VISHWAKARMA RAM ASREY, JAIN SHREYANS KUMAR, BHARATE SANDIP BIBISHAN, DAR ABID HAMID, KHAJURIA ANAMIKA, MEENA	0037NF2013/EP	14724520.3 & date 12-10-2015	

		SAMDARSHI, BHOLA SUNIL KUMAR, QAZI ASIF KHURDHID, HUSSAIN AASHIQ, SIDIQ TABASUM, UMA SHAANKER RAMANAN, RAVIKANTH GUDASALAMANI, VASUDEVA RAMESH, MOHANA KUMARA PATEL, GANESHAIAH KOTIGANAHALLI			
7	ROHITUKINE ANALOGS AS CYCLIN-DEPENDENT KINASE INHIBITORS AND A PROCESS FOR THE PREPARATION THEREOF	VISHWAKARMA RAM ASREY, BHARATE SANDIP BIBISHAN, BHUSHAN SHASHI, MONDHE DILIP MANIKRAO, JAIN SHREYANS KUMAR, MEENA SAMDARSHI, GURU SANTOSH KUMAR, PATHANIA ANUP SINGH, KUMAR SURESH, BEHL AKANKSHA, MINTOO MUBASHIR JAVED, BHARATE SONALI SANDIP, JOSHI PRASHANT	0219NF2012/US	14/784489 & date 14-10-2015	
8	ROHITUKINE ANALOGS AS CYCLIN-DEPENDENT KINASE INHIBITORS AND A PROCESS FOR THE	VISHWAKARMA RAM ASREY, BHARATE SANDIP BIBISHAN, BHUSHAN SHASHI, MONDHE DILIP	0219NF2012/EP	14734915.3 & date 14-10-2015	

	PREPARATION THEREOF	MANIKRAO, JAIN SHREYANS KUMAR, MEENA SAMDARSHI, GURU SANTOSH KUMAR, PATHANIA ANUP SINGH, KUMAR SURESH, BEHL AKANKSHA, MINTOO MUBASHIR JAVED, BHARATE SONALI SANDIP, JOSHI PRASHANT				
9	10-SUBSTITUTED COLCHICINOIDS AS POTENT ANTICANCER AGENTS	VISHWAKARMA RAM, BHARATE SANDIP BIBISHAN, KUMAR AJAY, SINGH BALJINDER, KUMAR ASHOK, BHUSHAN SHASHI, HAMID ABID, JOSHI PRASHANT, GURU SANTOSH KUMAR, KUMAR SURESH, HUSSAIN AASHIQ, QAZI ASIF KHURSHID, BHARATE SONALI SANDIP, SHARMA PARDUMAN, SAXENA AJIT KUMAR, MONDHE DILIP MANIKRAO, MAHAJAN GIRISH, WANI ZAHOOR	0059NF2014/WO	PCT/IN2015/050135 & date 14-10-2015		- - -
10	A	VISHWAKARM	0036NF20	PCT/IN2015/050		-

	PHARMACEUTICAL COMPOSITION FOR THE TREATMENT OF MULTI-DRUG RESISTANT INFECTIONS	A RAM, KUMAR AJAY, KHAN INSHAD ALI, BHARATE SANDIP BIBISHAN, JOSHI PRASHANT, SINGH SAMSHER, SATTI NARESH	14/WO	143 & date 21-10-2015		
11	N-SUBSTITUTED BETA-CARBOLINIUM COMPOUNDS AS POTENT P-GLYCOPROTEIN INDUCERS	BHARATE SANDIP, KUMAR AJAY, MANDA SUDHAKAR, JOSHI PRASHANT, BHARATE SONALI, VISHWAKARMA RAM	0302NF2013/WO	PCT/IN2015/050142 & date 21-10-2015		
12	TETRAHYDRO-2H-PYRANO [3,2-C] ISOCHROMENE-6-ONES AND ANALOGS FOR THE TREATMENT OF INFLAMMATORY DISORDERS	JAIN SHREYANS KUMAR, SIDIQ TABASUM, MEENA SAMDARSHI, KHAJURIA ANAMIKA, VISHWAKARMA RAM ASREY, BHARATE SANDIP BIBISHAN	0063NF2012/EP	13819083.0 & date 06-11-2015		
13	TETRAHYDRO-2H-PYRANO [3,2-C] ISOCHROMENE-6-ONES AND ANALOGS FOR THE TREATMENT OF INFLAMMATORY DISORDERS	JAIN SHREYANS KUMAR, SIDIQ TABASUM, MEENA SAMDARSHI, KHAJURIA ANAMIKA, VISHWAKARMA RAM ASREY, BHARATE SANDIP BIBISHAN	0063NF2012/US	14/891,706 & date 17-11-2015		
14	NOVEL 1,3,5 - TRIAZINE BASED PI3K INHIBITORS AS ANTICANCER AGENTS AND A PROCESS FOR THE PREPARATION THEREOF	THATIKONDA THANUSHA, KUMAR SURESH, SINGH UMED, MAHAJAN PRIYA, MAHAJAN GIRISH, NARGOTRA	0127NF2014/WO	PCT/IN2015/050169 & date 17-11-2015		

		AMIT, MALIK FAYAZ, MONDHE DILIP MANIKRAO, VISHWAKARMA RAM ASREY, SINGH PARVINDER PAL				
15	TETRAHYDRO-2H-PYRANO [3,2-C] ISOCHROMENE-6-ONES AND ANALOGS FOR THE TREATMENT OF INFLAMMATORY DISORDERS	JAIN SHREYANS KUMAR, SIDIQ TABASUM, MEENA SAMDARSHI, KHAJURIA ANAMIKA, VISHWAKARMA RAM ASREY, BHARATE SANDIP BIBISHAN	0063NF2012/CA	2,913,281 & date 23-11-2015		
16	Brachiatin D and process for their production thereof	DEEPIKA SINGH, JAI PRAKASH SHARMA, SUNDEEP JAGLAN, ABID HAMID DAR, ANAMIKA KHAJURIA, VARUN PRATAP SINGH, RAM ASREY VISHWAKARMA	0038NF2013/EP	14790347.0 & date 23-02-2016		
17	Brachiatin D and process for their production thereof	DEEPIKA SINGH, JAI PRAKASH SHARMA, SUNDEEP JAGLAN, ABID HAMID DAR, ANAMIKA KHAJURIA, VARUN PRATAP SINGH, RAM ASREY VISHWAKARMA	0038NF2013/US	14/914,094 & date 24-02-2016		

C. Patents Granted in India

SNo	Title	Inventors	NFNO	Application No.	Grant Date	Patent No.
1	A PHARMACEUTICAL	QAZI GHULAM	0151NF2006/IN	0570DEL2007	31/Aug/2015	268507

	COMPOSITION FOR THE TREATMENT OF CANCER AND RELATED DISORDERS	NABI, TANEJA SUBHASH CHANDRA, SINGH JASWANT, SAXENA AJIT KUMAR, SETHI VIJAY KUMAR, MONDHE DILIP MANIKRAO, KA		& date 16-03-2007		

D. Patents Granted in Abroad

SNo	Title	Inventors	NFNO	Application No.	Grant Date	Patent No.
1	Aromatic amides as potentiators of bioefficacy of anti-infective drugs	Koul; Surrinder (Jammu Tawi, IN), Koul; Jawahir Lal (Jammu Tawi, IN), Taneja; Subhash Chandra (Jammu Tawi, IN), Gupta; Pankaj (Jammu Tawi, IN), Khan; Inshad Ali (Jammu Tawi, IN), Mirza; Zahid Mehmood (Jammu Tawi, IN), Kumar; Ashwani (Jammu Tawi, TW), Johri; Rakesh Kamal (Jammu Tawi, IN), Pandita; Monika (Jammu Tawi, IN), Khosa; Anita (Jammu Tawi, IN), Tikoo; Ashok Kumar (Jammu Tawi, IN), Sharma; Subhash Chander (Jammu Tawi, IN), Verma; Vijeshwar (Jammu Tawi, IN), Qazi; Ghulam Nabi (Jammu Tawi, IN)	0472NF2004/US	14/051671 & date 11-10-2013	07/Jul/2015	9073860
2	A PROCESS FOR THE PREPARATION OF OPTICALLY ACTIVE N-BENZYL-3-	SUBHASH CHANDRA TANEJA, MUSHTAQ	0159NF2008/JP	2011-537014 & date 24-05--2011	17/Jul/2015	5779100

	HYDROXYPYRROLIDINES	AHMAD AGA, BRIJESH KUMAR, VIJAY KUMAR SETHI, SAMAR SINGH ANDOTRA, GHULAM NABI QAZI				
3	QUINOLYLPIPERAZINO SUBSTITUTED THIOLACTONE COMPOUNDS AND PROCESS FOR THE PREPARATION THEREOF	AHMED KAMAL, SHAIK AZEEZA, AHMED ALI SHAIK, M SHAHEER MALIK, INSHAD ALI KHAN, SHEIKH TASDUQ ABDULLAH, SANDEEP SHARMA, ANSHU BEULAH RAM	0073NF2010/US	13/643133 & date 10-04-2013	21/Jul/2015	9085557
4	CHALCONES AS ENHANCER OF ANTIMICROBIAL AGENTS	SUBRAMANYAM Ravi, DU-THUMM Laurence, QAZI Ghulam Nabi, KHAN Inshad Ali, SURI Krishan Avtar, SATTI Naresh Kumar, ALI Furqan, KALIA Nitin Pal	0047NF2014/US	13/515836 & date14-06-2012	24/Nov/2015	9192589
5	DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF ISOFORM SELECTIVE ANALOGS OF LIPHAGANE SCAFFOLD AS ANTICANCER AGENTS: P13K-ALPHA/BETA INHIBITORS	RAM A VISHWAKARMA, SANGHAPAL DAMODHAR SAWANT, PARVINDER PAL SINGH, ABID HAMID DAR, PARDUMAN RAJ SHARMA, AJIT KUMAR SAXENA, AMIT NARGOTRA, KOLLURU ANJANEYA ARAVIND KUMAR, MUDUDUDDLA RAMESH, ASIF KHURSHID QAZI, AASHIQ HUSSAIN, NAYAN CHANAURIA	0195NF2011/US	14/385808 & date 17-09-2014	08/Dec/2015	9206201

6	SYNERGISTIC TISSUE CULTURE MEDIA FORMULATION FOR IN VITRO REGENERATION OF SWERTIA CHIRATA HAM	AHUJA A, KOUL S, KAUL BL, VERMA NK, KAUL MK, RAINA RK, QAZI GN	0415NF2001/DE	10197282.2 & date 26-05-2004	31/Dec/2015	1019728 2

BOOKS CHAPTER

- Release a booklet of Medicinal & Aromatic plants covering the cultivation, processing and marketing of major IIIM developed agrotechnologies of medicinal and aromatic plants on the occasion of Kisan Mela, Entrepreneurship Programme & Flower Show-2016 on March 13, 2016

- Ajai Prakash Gupta, Pankaj Pandotra, ManojKushwaha, Rajni Sharma and Suphla Gupta (2015). Alkaloids: A Source of Anticancer Agents from Nature. Studies in Natural Products Chemistry, volume 46; 341-445. ISBN: 978-0-444-63462-7

- Saima Khan, Pankaj Pandotra, Asif Khan, Sajad A Lone, Malik Muzafar, Ajai P Gupta and Suphla Gupta (2016). ACCEPTED. Medicinal and nutritional qualities of Zingiber officinalein Health Fruits, Vegetables, and Herbs: Bioactive Foods in Promoting. Edited by Ronald Watson and Victor Preedy.Elsevier The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, United Kingdom, Registration No. 1982084, Registered in England and Wales.

- Singh Bikarma (2015) Himalayan Orchid: distribution and taxonomy. Published by Educationist Press, Write & Print Publications, New Delhi, India. 224 pages [ISBN: 978-93-84649-11-1].

INVITED TALKS / SEMINARS / CONFERENCES / WORSHOPS SYMPOSIUM / POSTER PRESENTATIONS

- Radio talk on 'Cloning: sambhavnaiyeaurChunautiyaan"-All India Radio, Jammu & Kashmir on Feb 5, 2016.
- Radio talk on 'sukshmjeevokivividhduniya'-All India Radio, Jammu & Kashmir on July, 21 2015.
- Radio talk on 'Vigyan Mein Mahilaye'- All India Radio, Jammu & Kashmir on Feb 19, 2015
- Invited talk entitled "*Orchid Gem in Indian Himalaya*" delivered in UGC sponsored refresher course"; held at Department of Botany, University of Jammu, J&K State on 23rd November, 2015.
- Invited talk entitled "*Science of Indian Orchid: History, Current Perspective and Future Directions*" delivered in a National Seminar on New Vistas in Plants and Microbial Sciences 11-12 March, 2016"; held at Department of Botany, University of Jammu, J&K State on 12th March, 2016.
- Invited talk entitled "*Field identification and herbarium techniques for higher plants*" delivered in a *Taxonomic Workshop on Different Plant Groups*; Jointly organized by DEER and IIIM under the aegis of NASI (J&K Chapter) on 14th March, 2016.
- Invited lecture on, **"**HPLC and GLC**"**, at Govt. College for Women,

Gandhi Nagar, Jammu on 28 and 29th April 2015.

- Invited lecture on, "Quality assessment of essential oils using modern tools", in "Training cum Workshop on Essential Oils, Perfumery & Aromatherapy" held at FRI , Dehradun from June 22-26, 2015.
- Invited Lecture, "Patent, Copyright, Trademark & Infringement", Worksh op on "Intellectual Property Right (IPR)", Govt. College for Women, Gandhi Nagar, Jammu, 18th November 2015.
- Chief Guest and Inaugural Lecture, **"**Biodiversity and Medicinal Plants", National Seminar on Environment: Issues & strategies, R. S. S. College, Kanpur, 28th Jan 2016.
- Invited Guest Speaker, **"**Chromolith RP-18e Muti-utility column**"**, Chromatography ENGAGE" Merck, India at New Delhi, 24th Feb 2016.
- Invited lecture and jury member, **"**Innovative Ideas from Every Day Lives", National Science Day (Innovation & make in India Fair), Org. by National Council for Science & Technology Communication (DST), New Delhi & J & K State Science Technology & Innovati on Council, 25-26th Feb. 2016 at GCW, Gandhi Nagar, Jammu
- Invited Lecture, **"**Analytical Techniques HPLC AND HPTLC**"**, **"**Faculty Development Programme On Current Scenario & Future Perspective's In Pharmaceutical Research", Khalsa College Of Pharmacy, Gurusar Sadhar, Ludhiana, 8th to 12th March 2016.
- Guest of Honour "World Health Day", Organised by Indo-Vietnam Medicine, 7th April 2016, K. L. Saigal Memorial Auditorium, Jammu.
- Participated in Brainstorming session on "Climate Change" during 19-20 May 2015 under the umbrella of NASI, J&K Chapter, at CSIR-IIIM
- Participated in Brainstorming session on "New Bioresources for high value foods and nutraceutical products" and delivered talk on 'Microorganisms as potential source of polyunsaturated fatty acids' on 15th September, 2015
- Delivered an invited talk on "Development and In-Vitro evaluation of dendrimer based formulation of Erlotiniib HCl" in 3rd International Conference on Nanostructured Materials and Nanocomposites (ICNM-2015) held at Hindustan College of Science and Technology, Farah (Mathura) U.P. India, during Dec.12, 2015, to Dec.14, 2015.
- Participated in exhibiting CSIR-IIIM displays on National Science Day, 27 February, 2016 under the umbrella of NASI, J&K Chapter, at Katra
- Participated in exhibiting CSIR-IIIM displays during Kisan Mela, Entrepreneurship Programme & Flower Show' on March 13, 2016 at CSIR-IIIM farm, Chatha.
- Training-cum-demonstration programme were organized at Basohli, Kathua (J&K), 25 July, 2015. In this programme, CSIR-IIIM formally launched the JAAG project for cultivation of CSIR crops in 10 districts of Jammu Division.

About 250 farmers, growers, entrepreneurs were participated in the programme.

- CSIR-IIIM organized one day training programme on "Cultivation, Processing and Marketing of Medicinal & Aromatic Plants" on September 2, 2015 at IIIM Farm, Chatha under JAAG project. In this programme, 20 elite farmers from 6 districts were participated for training and demonstration of MAPs selected for plantation under JAAG project.
- CSIR-IIIM organized one day training programme on "Cultivation, Processing and Marketing of Medicinal and Aromatic Plants" under JAAG project at Kalra College of Education, Udhampur on November 17, 2015. In this training programme, more than 200 farmers and entrepreneurs of Udhampur and Ramban Districts were participated. CSIR-IIIM scientists delivered lectures and displayed medicinal and aromatic plants, their cultivation technologies for awareness of farmers and also demonstrated the mobile distillation unit for extraction of essential oils for the benefit of farmers.
- Organized one day Kisan Mela, Entrepreneurship Programme & Flower Show at IIIM farm, Chatha on March 13, 2016. About 500 farmers, growers, entrepreneurs, students, Army personnel were participated.
- Organize a "National Seminar on Aromatic Oil RRL-CN-5 Project" in collaboration with Pacific Agro, Nagpur at CSIR-NEERI, Nagpur.
- Organized training-cum-awareness programmes in different locations like Samba, Kathua, R. S. Pura, Reasi, Udhampur of Jammu region under JAAG project. In which more than 700 farmers, growers awared about cultivation, processing and marketing of aromatic crops

AWARDS

Dr. Neha Dhar: Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, Singapore 117604.

Dr. Satiander Rana: Genetics, Development & Cell Biology, NSF Engineering Research Center for Bio-renewable Chemicals, Biorenewables Research Laboratory, Iowa State University Ames, IA 50011, USA.

Dr. Wajid Waheed Bhat: Biotransformation, Scion Research NZ-Crown Research Institute, 49 Sala Street, Rotorua 3046, New Zealand.

Dr. Sumeer Razdan: Nutritional of Crops Group, Plant Biology, ICGEB, Aruna Asif Ali Marg, New Delhi, 110067

Farnaz Yusuf has been awarded Ph.D. for her work entitled "Purification and Characterization of Nitrilase from *Fusarium proliferatum* and its Pharmaceutical Applications" by Guru Nanak Dev University, Amritsar **(2016)**

RESEARCH COUNCIL 2015-2016

Chairman			
1	**Prof. Goverdhan Mehta** National Research Proffessor and Lily- Jubilant Chair School of Chemistry University of Hyderabad Hyderabad – 500046		
External Members			
2	**Dr. Rajiv I. Modi** Managing Director Cadila Pharmaceuticals Ltd. Cadila Corporate Campus Sarkhej Dhokla Road, Bhat Ahmedabad - 382210	3	**Prof. Sudhir Kumar Sopory** Vice Chancellor Jawaharlal Nehru University New Delhi-110067
4	**Prof. Satyajit Mayor** Professor and Dean National Centre for Biological Sciences (Tata Institute of Fundamental Research) Bellary Road, GKVK Campus Bengaluru-560065	5	**Prof. Y.K. Gupta** Professor and Head, Department of Pharmacology All India Institute of Medical New Delhi – 110029
6	**Dr. Satyajit Rath** Scientist National Institute of Immunology Aruna Asaf Ali Marg New Delhi – 110067	7	**Dr. T.S. Balganesh** CSIR Distinguished Scientist CSIR- Fourth Paradigm Institute NAL Belur Campus Bengaluru - 560037
Agency Representative		**DG Nominee**	
8	**Dr. G . J Samathanam** Adviser Department of Science and Technology Technology Bhawan, New Mehrauli Road New Delhi-110016	9	**Dr. P . K Biswas** Former Adviser (S&T), Planning Commissiong MS-11/905, Kendriya Vihar, Sector-56 Gurgaon-122003
Sister Laboratory		**Cluster Director**	
10	**Dr. Ramesh V.Sonti** Scientist CSIR- Center for Cellular and Molecular Biology, Uppal Road Hyderabad – 500007	11	**Dr. P . S Ahuja** Director CSIR- Institute of Himalayan Bioresource Technology Post Box No. 6 Palampur – 176061
Director		**Permanent Invitee**	
12	**Dr. Ram Vishwakarma** Director CSIR- Indian Institute of Integrative Medicine Canal Road, Jammu Tawi - 180001	13	**Head or his Nominee** Planning & Performance Division Council of Scientific & industrial Research Anusandhan Bhawan, 2, Rafi Marg New Delhi- 110001

MANAGEMENT COUNCIL 2015 – 2016

Dr. Ram Vishwakarma — **Chairman**
Director, Indian Institute of Integrative Medicine
Canal Road, Jammu

Dr. Girish Sahani — **Member**
Director, Institute of Microbial Technology
Chandigarh

Er. Abdul Rahim — **Member**
Principal Scientist /Head, PME Division
Indian Institute of Integrative Medicine, Canal Road, Jammu

Er. Rajneesh Anand — **Member**
Sr. Principal Scientist
Indian Institute of Integrative Medicine, Canal Road, Jammu

Dr. Parthasarthi Das — **Member**
Principal Scientist
Indian Institute of Integrative Medicine, Canal Road, Jammu

Dr. Dhiraj Kumar Vyas — **Member**
Scientist
Indian Institute of Integrative Medicine, Canal Road, Jammu

Dr. Shashank Kumar Singh — **Member**
Sr. Scientist
Indian Institute of Integrative Medicine, Canal Road, Jammu

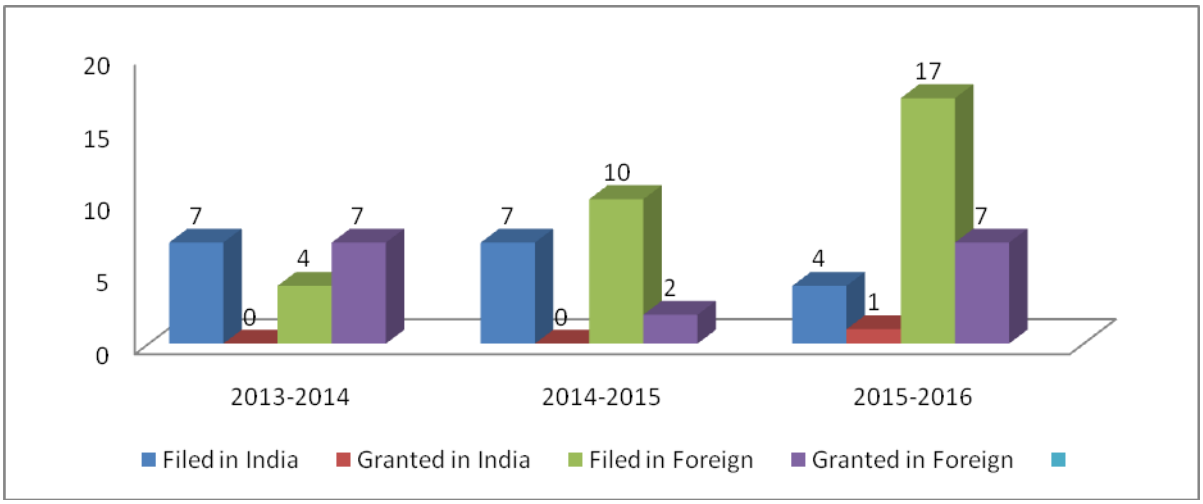
Sh. R K Raina — **Member**
F&AO
Indian Institute of Integrative Medicine, Canal Road, Jammu

Sh.Pankaj Bhadur — **Member**
COA
Indian Institute of Integrative Medicine, Canal Road, Jammu

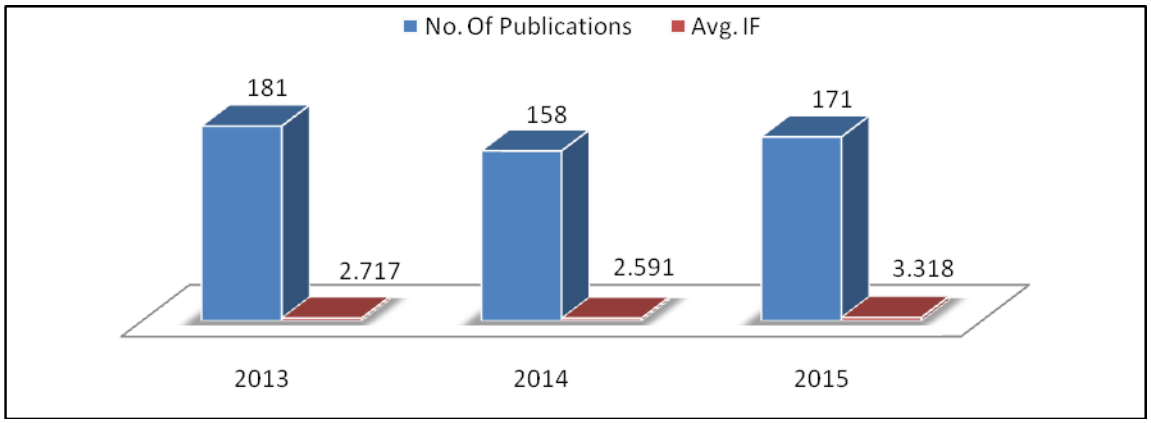
Dr. S.C. Sharma — **Member**
Principal Technical Officer
Indian Institute of Integrative Medicine, Canal Road, Jammu.

PERFORMANCE PARAMETERS

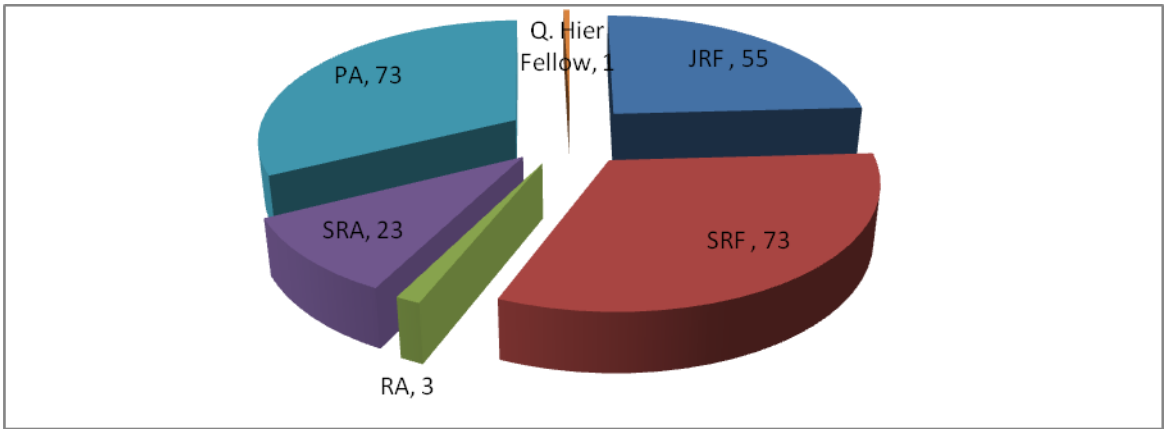
Patents



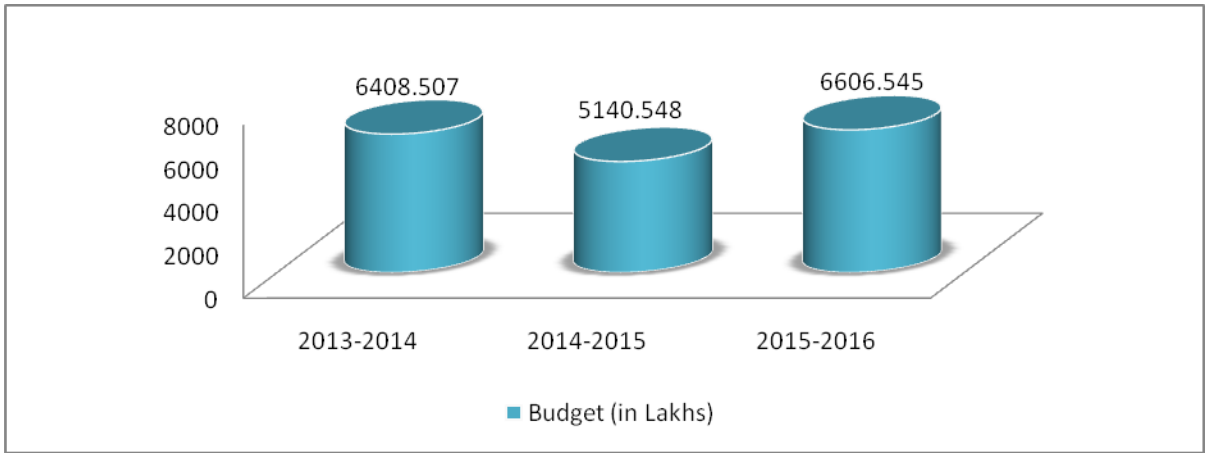
Publications [Calender Year]



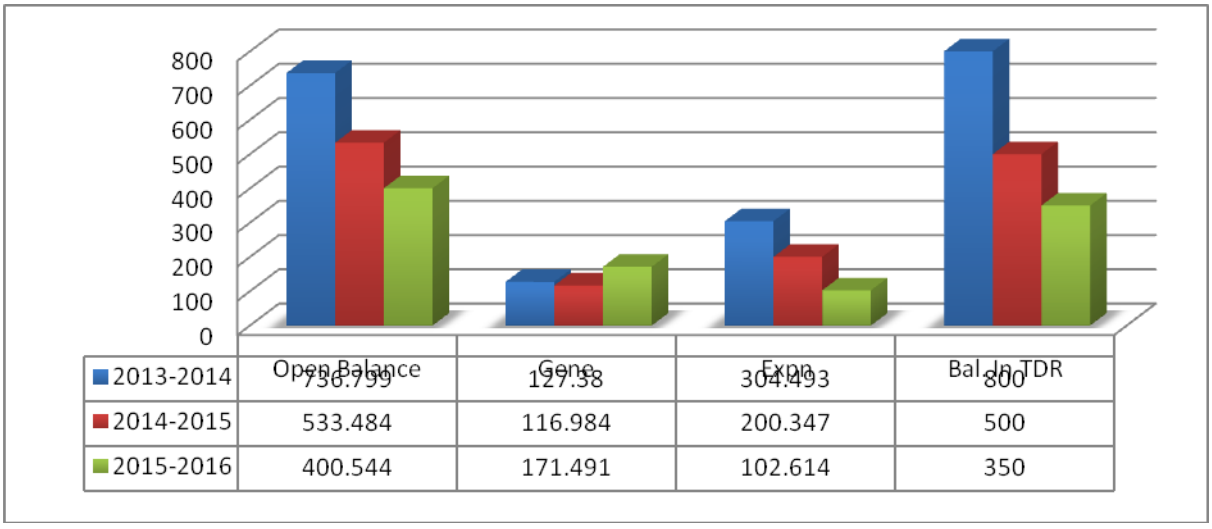
Fellows



Budget (Rs. In Lakhs)

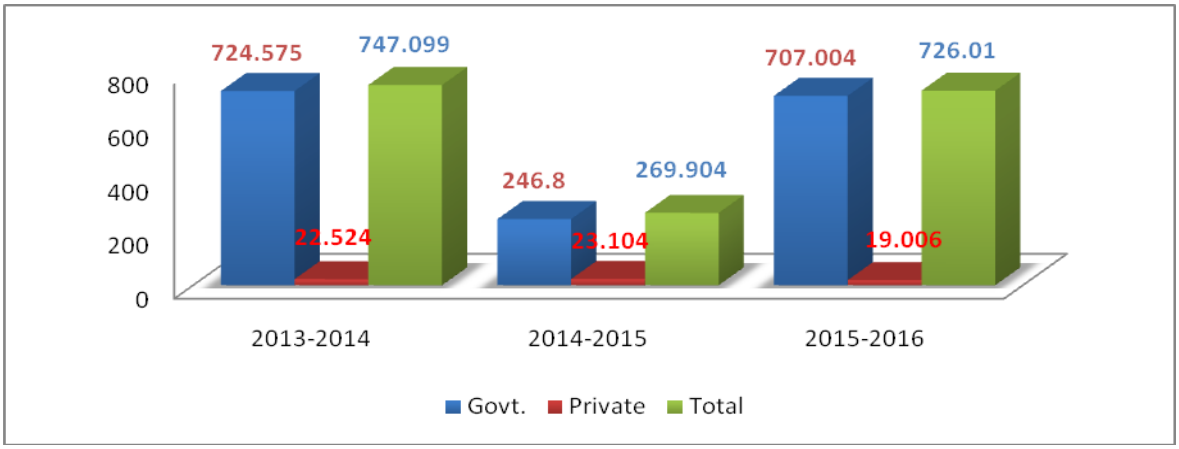


Institute's Reserve (Rs. In Lakhs)



	Open Balance	Gene	Expn	Bal. In TDR
2013-2014	736.799	127.58	304.493	800
2014-2015	533.484	116.984	200.347	500
2015-2016	400.544	171.491	102.614	350

External Cash Flow



RURAL DEVELOPMENT AND SOCIETAL ACTIVITIES

Covered about >50.0 hectare of cultivated land with cultivation of CSIR agrotechnologies (*Cymbopogon* sps., Mint sps., Lavender, Rose, Rosemary, *Monarda citriodora*) for better economical return over traditional crops. Ten districts Kathua, Udhampur, Reasi, Ramban, Poonch, Doda, Rajouri, Kishtwar Jammu, Samba of J&K is covered under the project. More than 150 nos. of farmers beneficiaries are registered under the project.

- Development of 3000 nos. of Aroma Value Kits for commercial use.

- Development of Phalsa Health Drink (Shivalik). Developed 30,000 of tetra pack for market evaluation.

SC/ST/OBC REPORT-I

ANNUAL STATEMENT SHOWING THE REPRESENTATION OF SCs, STs AND OBCs AS ON FIRST JANUARY OF THE YEAR AND NUMBER OF APPOINTMENTS MADE DURING THE PRECEDING CALENDER YEAR 2015

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH (DSIR)
O/o INDIAN INSTITUTE OF INTEGRATIVE MEDICINE, JAMMU

	Representation of SCs/STs/OBCs (As on 01.01.2016)				Number of appointments made during the calendar year 2015									
					By Direct Recruitment				By Promotion			By Deputation		
Groups	Total number of Employees	SCs	STs	OBCs	Total	SCs	STs	OBCs	Total	SCs	STs	Total	SCs	STS
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Group A	95	12	03	05	03	-	-	-	-	-	-	-	-	-
Group B	96	22	01	09	-	-	-	-	01	-	-	-	-	-
Group C	92	39	01	03	-	-	-	-	-	-	-	-	-	-
Group D (Excluding Sweepers)														
Group D (Sweepers)														
TOTAL														

SO (Estb)
O/o Indian Institute of Integrative Medicine, Jammu- 180001

SC/ST/OBC REPORT-II

ANNUAL STATEMENT SHOWING THE REPRESENTATION OF SCs, STs AND OBCs IN VARIOUS GROUP'A' SERVICES AS ON FIRST JANUARY AND NUMBER OF APPOINTMENTS MADE IN THE SERVICE IN VARIOUS GRADES IN THE CALENDER YEAR 2015

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH (DSIR)
O/o INDIAN INSTITUTE OF INTEGRATIVE MEDICINE, JAMMU

	Representation of SCs/STs/OBCs (As on 01.01.2016)				Number of appointments made during the calendar year 2015									
					By Direct Recruitment				By Promotion			By Deputation		
Pay Band and Grade Pay	Total number of Employees	SCs	STs	OBCs	Total	SCs	STs	OBCs	Total	SCs	STs	Total	SCs	STS
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PB-3 Rs.5400	06	01	01	-	-	-	-	-	-	-	-	-	-	-
PB-3 Rs.6600	25	07	-	04	01	-	-	-	-	-	-	-	-	-
PB-3 Rs.7600	34	02	-	01	02	-	-	-	-	-	-	-	-	-
PB-4 Rs.8700	28	01	1	-	-	-	-	-	-	-	-	-	-	-
PB-4 Rs.8900	02	-	1	01	-	-	-	-	-	-	-	-	-	-
PB-4 Rs.10,000	02	01	-	-	-	-	-	-	-	-	-	-	-	-
HAG+Above														
TOTAL	97	12	03	06	03									

SO (Estb)
O/o Indian Institute of Integrative Medicine, Jammu- 180001

PWD Report I

ANNUAL STATEMENT SHOWING THE REPRESENTATION OF THE PERSONS WITH DISABILITIES IN SERVICES (AS ON 1ST JANUARY 2016)

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH (DSIR)
O/o INDIAN INSTITUTE OF INTEGRATIVE MEDICINE, JAMMU

Group	Number of Employees				
	Total	In Identified posts	VH	HH	OH
1	2	3	4	5	6
Group A	95	03 (2OH;1HH)	-	-	01*
Group B	96	02 (1VH;1HH)	-	-	02
Group C	92	01 (1OH;1HH)	-	-	01**
Group D					
TOTAL					

Note: (i) VH stands for Visually Handicapped (persons suffering from blinders or low vision).
(ii) HH stands for Hearing Handicapped (persons suffering from hearing impairment).
(iii) OH stands for Orthopaedically Handicapped (persons suffering from locomotor disability or cerebral palsy).

- *** Two post one each under OH and HH are still lying vacant**
- **** One post under HH is lying waste.**

SO (Estb)
O/o Indian Institute of Integrative Medicine, Jammu - 180001

PWD REPORT II

STATEMENT SHOWING THE NUMBER OF PERSONS WITH DISABILITIES APPOINTED DURING THE YEAR
(As on 1st. January 2016)

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH (DSIR)
O/o INDIAN INSTITUTE OF INTEGRATIVE MEDICINE, JAMMU

GROUP	DIRECT RECRUITMENT								PROMOTION							
	No. of vacancies reserved			No. of Appointments Made					No. of vacancies reserved			No. of Appointments Made				
	VH	HH	OH	Total	In Identified Posts	VH	HH	OH	VH	HH	OH	Total	In Identified Posts	VH	HH	OH
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Group A																
Group B																
Group C																
Group D																

Note: (i) VH stands for Visually Handicapped (persons suffering from blinders or low vision).
(ii) HH stands for Hearing Handicapped (persons suffering from hearing impairment).
(iii) OH stands for Orthopaedically Handicapped (persons suffering from locomotor disability or cerebral palsy).
(IV) There is no reservation for persons with disabilities in case of promotion to Group A and B posts. However, persons with disabilities can be promoted to such posts, provided the concerned post is identified suitable for persons with disabilities.

Note: No direct recruitment/ promotion under VH/HH/OH have been made in this institute during 2015 hence NIL report in this regard may be considered from this institute.

SO (Estb)
O/o IIIM, Jammu - 180001

KNOWLEDGE RESOURCE CENTRE

Introduction

Library in this campus was in existence even during pre-independence days. During those times, it was known as 'Drug Research Laboratory (DRL) - Library' which was renamed as 'Regional Research Laboratory (RRL) - Library' in 1957 when CSIR took-over DRL and renamed the Institution as 'Regional Research Laboratory (RRL)'. Library shifted to its new building (present building) on 13th September, 1974. Subsequently, with the renaming of RRL as 'CSIR-Indian Institute of Integrative Medicine (IIIM)' and renaming of CSIR Libraries as 'S&T Knowledge Resource Centres', it is presently known as "IIIM S&T Knowledge Resource Centre (KRC)."

Objectives:

The objectives of IIIM-KRC are to further the interests of 'Users' by providing them library services to enable them to keep a track of significant development in their fields of interest. It supports its Scientists, Students and other S&T users with current and even evolving knowledge in their respective spheres of R&D activities.

Membership:

IIIM KRC caters to the information requirements of not only internal users but also of external users, like - postgraduate students, faculty members of colleges & universities; and corporate members. However, the membership for external users is on nominal payment basis.

Collection:

a) Print Collection:

Over the decades, IIIM has developed its rich Library resources. It has more than 100 year old rare research documents in its collection. It has grown into one of the most valuable research library in the country. It has a rich collection of books, periodicals, databases and other intellectual material. Broadly speaking, its collection covers subject areas of - Biotechnology, Botany, Medicinal Chemistry, Natural Products Chemistry (NPC), Pharmacology, Quality Control and Agrotechnology & Cultivation of Medicinal and Aromatic plants. During financial year 2015-16, IIIM KRC added 338 numbers of books and reference resource, including books in Hindi language, in its collection.

The present holding status is as under:
No. of purchased documents: 27457
No. of Periodicals Bound Volumes: 17187

b) **E-Resources:**

IIIM is an important member of 'National Knowledge Resource Consortium (NKRC)'. Through this consortium, KRC provides access to thousands of E- journals published by various publication groups - like American Chemical Society, Emerald, IEEE, JCCC, Nature Publishing Group, Oxford University Press, Royal Society of Chemistry, Taylor and Francis, Wiley, etc. It also subscribes other e-resources

which are not available through NKRC. Presently, a total of 26 online e-Journals and six online databases are being subscribed.

The total budget allocation during the financial year 2015-16 was Rs.1.0 crore.

It has computerized all its in-house activities which are being maintained and updated on a regular basis. KRC 'Online Public Access Catalogue (OPAC)' and 'Digital Institutional Repository' are also developed.

Services for Users:

- Online access to e-journals;
- Electronic Document Delivery Service (EDDS);
- Information search and retrieval facility;
- Plagiarism detection service;
- Reprographic and print facility;

Other initiatives:

KRC has developed its website with the help of IIIM-IT Cell. It can be accessed on URL: www.onlinelibrary.iiim.res.in/. Besides other useful information and links, links to all the subscribed e-resources; NKRC resources, etc. are available through this website.

A screenshot of IIIM KRC website Homepage.

भारतीय समवेत औषध संस्थान, जम्मू में राजभाषा की प्रगति में हिन्दी के कार्यक्रम

दिनांक 09-10 जून, 2015 को दो दिवसीय राजभाषा सम्मेलन/ यूनिकोड/कंप्यूटर प्रशिक्षण कार्यक्रम

सीएसआईआर-भारतीय समवेत औषध संस्थान, जम्मू के तत्वावधान में दिनांक 09-10 जून, 2015 को दो दिवसीय राजभाषा सम्मेलन/यूनिकोड/कंप्यूटर अनुप्रयुक्त प्रशिक्षण कार्यक्रम का उद्घाटन संस्थान के निदेशक एवं अध्यक्ष, नगर राजभाषा कार्यान्वयन समिति, जम्मू के डॉ. राम विश्वकर्मा ने किया। इस अवसर पर श्री किशोर कुमार, महाप्रबंधक, भारतीय खाद्य निगम, क्षेत्रीय कार्यालय-I, श्री ओमप्रकाश, कार्यपालक निदेशक, एनएचपीसी लिमिटेड, क्षेत्रीय कार्यालय, जम्मू, श्री एन.ए. अजाद, शाखा प्रबंधक, स्टील अथॉर्टी ऑफ इंडिया लिमिटेड, जम्मू, श्री रामानुज देवनाथन, प्राचार्य, राष्ट्रीय संस्कृत संस्थानम्, जम्मू तथा नराकास के केन्द्रीय कार्यालयों/बैंकों/उपक्रमों के सभी कार्यालयाध्यक्ष/नोडल अधिकारी/हिन्दी अधिकारी/हिन्दी अनुवादक/राजभाषा अधिकारी/प्रिन्ट व इलैक्ट्रॉनिक मीडिया के सभी संवाददाता तथा अन्य गणमान्य व्यक्ति उपस्थित थे। श्री आर.एस.गौतम, उपनिदेशक (राजभाषा), एन.डी.आर. आई. करनाल (हरियाणा) ने अपना विचार व्यक्त करते हुए हिन्दी के अन्तरराष्ट्रीय स्वरूप पर अपने विचार व्यक्त किये।

श्री ओम प्रकाश, कार्यपालक निदेशक, एनएचपीसी लिमिटेड, क्षेत्रीय कार्यालय, जम्म एवं कश्मीर के द्वारा इस हिन्दी के प्रशिक्षण कार्यक्रम के लिए अध्यक्ष महोदय का धन्यवाद करते हुए कहा कि राजभाषा के प्रचार-प्रसार के लिए यह मंच बहुत ही सशक्त मंच है। उन्होंने अपील करते हुए कहा कि हिन्दी को राजभाषा के रूप में सम्मान एवं प्रोत्साहन देना चाहिए।

डॉ. सुरेन्द्र चन्द्र, मुख्य वैज्ञानिक ने अपने विचार व्यक्त करते हुए कहा कि हिन्दी के प्रयोग और प्रसार के लिए हम प्रतिबद्ध है और हमें आज का यह प्रशिक्षण कार्यक्रम बहुत ही उपयोगी लगा और इस कार्यक्रम से हम रचनात्मक दृष्टि से आगे बढ़ते हैं।

संस्थान के निदेशक एवं अध्यक्ष, नराकास, जम्मू के डॉ. राम विश्वकर्मा ने अपने विचार व्यक्त करते हुए कहा कि हिन्दी के प्रयोग एवं उसके कार्यान्वयन के लिए प्रोत्साहन देना हमरा प्रथम कर्तव्य है। उन्होंने कहा कि आज हिन्दी का प्रयोग पब्लिक सेक्टर से प्राइवेट सेक्टर में अधिक हो रहा है। यह प्रशिक्षण कार्यक्रम बहुत उपयोगी साबित हुआ है।

प्रशिक्षण कार्यक्रम में उपस्थित सदस्यों का स्वागत एवं संचालन संस्थान के वरि. हिन्दी अधिकारी एवं सदस्य-सचिव, नराकास, जम्मू के डॉ. अमर सिंह ने किया।

अन्त में धन्यवाद प्रस्ताव संस्थान के वरिष्ठ वैज्ञानिक डॉ. सुरेश चन्द्र, मुख्य वैज्ञानिक ने किया।

नगर राजभाषा कार्यान्वयन समिति, जम्मू की छमाही बैठक दिनांक 30 जून, 2015 को भारतीय समवेत औषध संस्थान, जम्मू के कान्फ्रेंस हॉल में सम्पन्न।

भारत सरकार, गृह मंत्रालय, राजभाषा विभाग के निर्देशानुसार नगर राजभाषा कार्यान्वयन समिति, जम्मू की छमाही बैठक दिनांक 30 जून, 2015 (मंगलवार) को अपराह्न 3.00 बजे भारतीय समवेत औषध संस्थान, जम्मू के कॉन्फ्रेंस हॉल में आयोजित हुई। बैठक की अध्यक्षता संस्थान के मुख्य वैज्ञानिक एवं नराकास अध्यक्ष डॉ. सुरेश चन्द्र ने की। इस अवसर पर श्री प्रमोद कुमार, उपनिदेशक (कार्या.) क्षेत्रीय कार्यान्वयन कार्यालय दिल्ली, भारत सरकार, गृह मंत्रालय, राजभाषा विभाग, श्री किशोर कुमार, महा प्रबंधक, भारतीय खाद्य निगम, क्षेत्रीय कार्यान्वयन कार्यालय, जम्मू, श्री डी.के.गौतम, निदेशक, भारतीय विमानपत्तन प्राधिकरण, जम्मू, श्री पंकज बहादुर, प्रशासनिक अधिकारी, भारतीय समवेत औषध संस्थान, जम्मू, श्री उमंग मैनी, मुख्य प्रबंधक, पंजाब नेशनल बैंक, क्षेत्रीय कार्यालय, जम्मू, डॉ. शरत चन्द, सहाचार्य, राष्ट्रीय संस्कृत संस्थान, श्री रणवीर परिसर कोट बलवाल, जम्मू तथा नगर जम्मू के केन्द्रीय कार्यालयों/बैंकों/उपक्रमों से आये सभी कार्यालय अध्यक्ष, हिन्दी अधिकारी/राजभाषा अधिकारी/नोडल अधिकारी/हिन्दी अनुवादक तथा प्रिन्ट एवं इलैक्ट्रॉनिक मीडिया के समस्त संवाददाता एवं अन्य गणमान्य व्यक्ति उपस्थित थे।

सर्वप्रथम बैठक में उपस्थित कार्यालय प्रमुखों एवं सज्जनों का स्वागत डॉ. अमर सिंह, वरि. हिन्दी अधिकारी एवं सचिव, नराकास, जम्मू ने किया। उन्होंने अपने स्वागत संबोधन में कहा कि इस बैठक में प्रथम अक्टूबर, 2014 से 31 मार्च, 2015 के दौरान प्राप्त तिमाही प्रगति रिपोर्टों की समीक्षा तथा आपके कार्यालय में राजभाषा हिन्दी में किये गये कार्यों की समीक्षा तथा इससे संबंधित आपके कार्यालयों में उत्पन्न समस्याओं पर चर्चा की जाएगी। संघ के विभिन्न राजकीय प्रयोजनों में इसके प्रगामी प्रयोग को बढ़ावा देने के लिए राजभाषा विभाग प्रति वर्ष एक वार्षिक कार्यक्रम जारी करता है, जिसके अनुसार हम कार्यालयों में राजभाषा के कार्य सम्पन्न करते हैं। चूंकि सरकारी कामकाज में मूल टिप्पण और प्रारूपण के लिए हिन्दी का ही प्रयोग किया जाए। जिसके अन्तर्गत धारा 3(3) का हम सबको अनुपालन सुनिश्चित करना चाहिए। यही संविधान की मूलभावना के अनुरूप होगा। सभी भारतीय भाषाएं देश की एकता की प्रतीक हैं। भारतीय संविधान में जो प्रावधान किये गये हैं इन आदेशों/अनुदेशों का पालन करना चाहिए और महामहिम राष्ट्रपति जी के संकल्पों का सम्मान करना चाहिए।

गत बैठक में नगर राजभाषा कार्यान्वयन समिति मंच के माध्यम से दिनांक 09-10 जून, 2015 को भारतीय समवेत औषध संस्थान, जम्मू के तत्वाधान में दो दिवसीय राजभाषा सम्मेलन/यूनिकोड/कंप्यूटर अनुप्रयुक्त प्रशिक्षण कार्यक्रम का उद्घाटन संस्थान के निदेशक एवं अध्यक्ष, नगर राजभाषा कार्यान्वयन समिति, जम्मू के डॉ. राम विश्वकर्मा ने किया। इस अवसर पर श्री किशोर कुमार, महाप्रबंधक, भारतीय खाद्य निगम, क्षेत्रीय कार्यालय-I, श्री ओमप्रकाश, कार्यपालक निदेशक, एनएचपीसी लिमिटेड, क्षेत्रीय कार्यालय, जम्मू, श्री एन.ए. अजाद, शाखा प्रबंधक, स्टील अथॉर्टी ऑफ इंडिया लिमिटेड, जम्मू, श्री रामानुज देवनाथन, प्राचार्य, राष्ट्रीय संस्कृत संस्थानम्, जम्मू तथा नराकास के केन्द्रीय कार्यालयों/बैंकों/उपक्रमों के सभी नोडल अधिकारी/हिन्दी अधिकारी/हिन्दी अनुवादक/राजभाषा

अधिकारी उपस्थित थे। सभी के विचार व्यक्त करते हुए उनके सौजन्य से राजभाषा सम्मेलन/भाषा कौशल/कम्प्यूटर प्रशिक्षण कार्यक्रम का सफल आयोजन किया गया। जिसमें नराकास, जम्मू के सभी सदस्य कार्यालयों के नोडल अधिकारी/राजभाषा अधिकारी/ हिन्दी अनुवादकों ने बढ़ चढ़कर भाग लिया और प्रतिभागियो को प्रमाण–पत्र एवं स्मृति चिन्ह अध्यक्ष महोदय के कर कमलों द्वारा प्रदान किये गये। कार्यक्रम में व्याख्याता के रूप में श्री आर.एस. गौतम, उपनिदेशक (राजभाषा), एन.डी.आर.आई. करनाल (हरियाणा) से उपस्थित थे। अन्य वक्ताओं को कम्प्यूटर के माध्यम से प्रशिक्षण के लिए स्लाइड पर प्रशिक्षण दिया।

संस्थान के मुख्य वैज्ञानिक डॉ. सुरेश चन्द्र ने बैठक में उपस्थित केन्द्रीय कार्यालय/बैंकों/उपक्रमों के सभी कार्यालय प्रमुखों एवं उपस्थित गणमान्य व्यक्तियों का अपने संस्थान की ओर से व नगर राजभाषा कार्यान्वयन समिति के मंच से स्वागत करते हुए अपने अध्यक्षीय संबोधन में कहा कि हिन्दी हमारे भारत की प्रमुख भाषा है, हमें इसका सम्मान करना चाहिए। हिन्दी हमारे सम्मान की प्रतीक है और यह एक अरब पच्चीस करोड़ भारतीयों की भाषा है। हिन्दी का प्रयोग भारत के अन्य पड़ोसी देशों में भी सहज रूप से हो रहा है। सूचना प्रौद्योगिकी के विकास में हिन्दी ही एक प्रमुख कारण है। उन्होंने कहा कि जब भारत का संविधान बना था तो यह मांग की गयी थी कि अंग्रेजी को राष्ट्रभाषा बनाया जाए, लेकिन डॉ. भीमराव अम्बेडकर इस बात पर सहमत नहीं हुए उनका स्पष्ट मत था कि हिन्दी देश की सर्वोपरि भाषा है। इसके अतिरिक्त कोई विकल्प स्वीकार नहीं होगा। भारत गाँव का देश है हमारे देश का प्रत्येक भारतीय हिन्दी के माध्यम से अपने विचार व्यक्त करते हैं और हिन्दी आज इलेक्ट्रॉनिक एवं प्रिन्ट मीडिया की प्रमुख भाषा है तथा विज्ञान को भी इसके माध्यम से व्यक्त किया जा सकता है। इसके लिए हम सबको अपना चिन्तन एवं मानसिकता को बदलना है। तभी देश में विज्ञान की प्रगति सम्भव है। उन्होंने सभी का हृदय से आभार सहित धन्यवाद किया।

अन्त में धन्यवाद प्रस्ताव संस्थान के प्रशासनिक अधिकारी, श्री पंकज बहादुर ने बैठक में अध्यक्ष महोदय एवं उपस्थित नराकास जम्मू के सभी केन्द्रीय कार्यालयों/बैंकों/उपक्रमों के कार्यालय प्रमुखों एवं नगर के प्रिन्ट व इलैक्ट्रानिक मीडिया के सभी संवाददाताओं का आभार व्यक्त करते हुए कहा कि बैठक में दूरदर्शन तथा मीडिया का सदैव सहयोग रहा है और बैठक के आयोजन में संस्थान के सभी संकाय सदस्यों ने सहयोग प्रदान किया। प्रबंधन के लिए संस्थान के वरि. हिन्दी अधिकारी एवं सदस्य सचिव, डॉ. अमर सिंह तथा समस्त स्टॉफ सदस्यों का आभार सहित धन्यवाद किया।

हिन्दी दिवस/सप्ताह, 2015 का कार्यक्रम

संघ की राजभाषा हिन्दी में सरकारी कामकाज तथा हिन्दी के प्रति रूचिजागृति करने के उद्देश्य से संस्थान में दिनांक 01-16 सितम्बर, 2015 के दौरान हिन्दी सप्ताह का आयोजन किया गया, जिसमें निबन्ध लेखन, श्रुतलेख, राजभाषा एवं विज्ञान प्रश्नोत्तरी, स्टॉफ सदस्यों के बच्चों के लिए सामान्य ज्ञान प्रतियोगिता, अनुवाद/टिप्पण एवं प्रारूपण, अन्तरविभागीय भाषण प्रतियोगिता आदि प्रतियोगिताएं आयोजित की गयीं। इसी उपलक्ष्य में दिनांक 08.09. 2015 (मंगलवार) को संस्थान के स्टॉफ सदस्यों के बच्चों के लिए सामान्य ज्ञान (जी.के.) प्रतियोगिता का आयोजन किया जा रहा है। जिसमें कक्षा 5 से 8 तक के बच्चों ने भाग लिया। इस दौरान हिन्दी के प्रयोग एवं प्रगति की दिशा में विभिन्न प्रतियोगिताओं में संस्थान के 320 स्टॉफ सदस्यों ने प्रतियोगी के रूप में प्रतिभागिता की, जिससे उनकी कार्य संस्कृति में इज़ाफा हुआ है और कुल 29 विजयी प्रतियोगियों को पुरस्कार दिये गये।

नगर राजभाषा कार्यान्वयन समिति, जम्मू कार्यालय को राजभाषा नीति के श्रेष्ठ निष्पादन के लिए 'प्रथम' पुरस्कार प्रदान किया गया है।

संस्थान के प्रशासनिक अधिकारी, श्री पंकज बहादुर शील्ड प्राप्त करते हुए साथ ही वरिष्ठ हिन्दी अधिकारी एवं सदस्य-सचिव डॉ. अमर सिंह प्रमाण पत्र प्राप्त करते हुए।

नगर राजभाषा कार्यान्वयन समिति, जम्मू कार्यालय को राजभाषा नीति के श्रेष्ठ निष्पादन के लिए वर्ष 2014-2015 के लिए **'प्रथम'** राजभाषा पुरस्कार दिनांक 16.10.2015 को गुरूनानक देव विश्वविद्यालय, अमृतसर के दसमेश ऑडिटोरियम में भारत सरकार, गृह मंत्रालय, राजभाषा विभाग के तत्वावधान में पंजाब के राज्यपाल माननीय प्रोफेसर कप्तान सिंह सोलंकी एवं सचिव, राजभाषा विभाग, नई दिल्ली द्वारा पुरस्कार प्रदान किया गया। यह पुरस्कार संस्थान के निदेशक, भारतीय समवेत औषध संस्थान, जम्मू एवं अध्यक्ष, नगर राजभाषा कार्यान्वयन समिति, जम्मू की ओर से संस्थान के प्रशासनिक अधिकारी, श्री पंकज बहादुर ने शील्ड प्राप्त की तथा प्रमाण पत्र वरिष्ठ हिन्दी अधिकारी एवं सदस्य-सचिव डॉ. अमर सिंह ने प्राप्त किया।

भारतीय समवेत औषध संस्थान, जम्मू कार्यालय को राजभाषा नीति के श्रेष्ठ निष्पादन के लिए 'द्वितीय' पुरस्कार प्रदान किया गया है।

संस्थान के प्रशासनिक अधिकारी, श्री पंकज बहादुर शील्ड प्राप्त करते हुए साथ ही वरिष्ठ हिन्दी अधिकारी एवं सदस्य-सचिव डॉ. अमर सिंह प्रमाण पत्र प्राप्त करते हुए।

भारतीय समवेत औषध संस्थान, जम्मू कार्यालय को राजभाषा नीति के श्रेष्ठ निष्पादन के लिए वर्ष 2014-2015 के लिए 'द्वितीय' राजभाषा पुरस्कार दिनांक 16.10.2015 को गुरूनानक देव विश्वविद्यालय, अमृतसर के दसमेश ऑडिटोरियम में भारत सरकार, गृह मंत्रालय, राजभाषा विभाग के तत्वावधान में पंजाब के राज्यपाल माननीय प्रोफेसर कप्तान सिंह सोलंकी एवं सचिव, राजभाषा विभाग, नई दिल्ली द्वारा प्रदान किया गया। यह पुरस्कार एवं शील्ड संस्थान के निदेशक, भारतीय समवेत औषध संस्थान, जम्मू की ओर से संस्थान के प्रशासनिक अधिकारी, श्री पंकज बहादुर ने शील्ड प्राप्त की तथा प्रमाण पत्र वरिष्ठ हिन्दी अधिकारी एवं सदस्य-सचिव डॉ. अमर सिंह ने प्राप्त किया।

हिन्दी टाइपिंग की एकदिवसीय कार्यशाला

भारतीय समवेत औषध संस्थान, जम्मू में संस्थान के अधिकारियों/कर्मचारियों को राजभाषा कार्यान्वयन में आने वाली कठिनाइयों एवं हिन्दी टाइपिंग में प्रशिक्षित करने हेतु श्री राकेश कुमार शर्मा, वरिष्ठ उपसचिव/वरिष्ठ प्रशासन नियंत्रक (सेवानिवृत्त), स्वच्छन्द राजभाषा सलाहकार और राजभाषा डिजिटल समाधान प्रदाता, दिल्ली द्वारा हमारे संस्थान में दिनांक 02 दिसम्बर, 2015 को 3.00 बजे कान्फ्रेंस हॉल में राजभाषा में कार्य करने एवं डिजिटल टूल्स के प्रयोग द्वारा हिन्दी टाइपिंग की एकदिवसीय कार्यशाला में विशिष्ट व्याख्यान प्रदान किया। इस कार्यक्रम में लगभग 100 अधिकारियों/कर्मचारियों ने प्रतिभागिता की और उनके द्वारा उपलब्ध कराए गए ज्ञान का लाभ उठाया ।

नगर राजभाषा कार्यान्वयन समिति, जम्मू की छमाही बैठक दिनांक 21 जनवरी, 2016 को सायं 3.00 बजे भारतीय समवेत औषध संस्थान, जम्मू के कान्फ्रेंस हॉल में सम्पन्न।

भारत सरकार, गृह मंत्रालय, राजभाषा विभाग के निर्देशानुसार नगर राजभाषा कार्यान्वयन समिति, जम्मू की छमाही बैठक दिनांक 21 जनवरी, 2016 (वृहस्पतिवार) को अपराह्न 3.00 बजे भारतीय समवेत औषध संस्थान, जम्मू के कॉन्फ्रेंस हॉल में आयोजित हुई। बैठक की अध्यक्षता संस्थान के मुख्य वैज्ञानिक एवं नराकास अध्यक्ष डॉ. सुरेश चन्द्र ने की। इस अवसर पर श्री एन.एस.मेहरा, अनुसंधान अधिकारी, क्षेत्रीय कार्यान्वयन कार्यालय दिल्ली, भारत सरकार, गृह मंत्रालय, राजभाषा विभाग, श्री आर.के.गुप्ता, मुख्य प्रबंधक, पंजाब नेशनल बैंक, प्रशासनिक कार्यालय, जम्मू, श्री सुधीर कुमार सिंह, मंडल यातायात प्रबंधक, उत्तर रेलवे, जम्मू, डॉ. शरत चन्द, सहाचार्य, राष्ट्रीय संस्कृत संस्थान, श्री रणवीर परिसर कोट भलवाल, जम्मू, श्री पंकज बहादुर, नियंत्रक प्रशासन, भारतीय समवेत औषध संस्थान, जम्मू तथा नगर जम्मू के केन्द्रीय कार्यालयों/बैंकों/उपक्रमों से आये सभी कार्यालय अध्यक्ष, हिन्दी अधिकारी/राजभाषा अधिकारी/नोडल अधिकारी/हिन्दी अनुवादक तथा प्रिन्ट एवं इलैक्ट्रॉनिक मीडिया के समस्त संवाददाता एवं अन्य गणमान्य व्यक्ति उपस्थित थे।

सर्वप्रथम बैठक में उपस्थित कार्यालय प्रमुखों एवं उपस्थित अधिकारियों का स्वागत डॉ. अमर सिंह, वरि. हिन्दी अधिकारी एवं सचिव, नराकास, जम्मू ने किया। उन्होंने अपने स्वागत संबोधन में कहा कि इस बैठक में प्रथम अप्रैल, 2015 से 30 सितम्बर, 2015 के दौरान प्राप्त तिमाही प्रगति रिपोर्टों की समीक्षा तथा आपके कार्यालय में राजभाषा हिन्दी में किये गये कार्यों की समीक्षा तथा इससे संबंधित कार्यालयों में उत्पन्न समस्याओं पर चर्चा की जाएगी। संघ के विभिन्न राजकीय प्रयोजनों में इसके प्रगामी प्रयोग को बढ़ावा देने के लिए राजभाषा विभाग प्रति वर्ष एक वार्षिक कार्यक्रम जारी करता है, जिसके अनुसार हम कार्यालयों में राजभाषा के कार्य सम्पन्न करते हैं। चूंकि सरकारी कामकाज में मूल टिप्पण और प्रारूपण के लिए हिन्दी का ही प्रयोग किया जाना होता है। जिसके अन्तर्गत धारा 3(3) का हम सबको अनुपालन सुनिश्चित करना चाहिए, यही संविधान की मूलभावना के अनुरूप होगा। सभी भारतीय भाषाएं देश की एकता की प्रतीक हैं। भारतीय संविधान में जो प्रावधान किये गये हैं इन आदेशों/अनुदेशों का पालन करना चाहिए और महामहिम राष्ट्रपति जी के संकल्पों का सम्मान करना चाहिए।

संस्थान के श्री पंकज बहादुर, नियंत्रक प्रशासन ने राजभाषा हिन्दी के प्रयोग एवं प्रसार के लिए महत्वपूर्ण सुझाव देते हुए कहा कि भारत सरकार, राजभाषा विभाग द्वारा जो आदेश/अनुदेश भेजे जाते हैं

और राजभाषा नीति के अन्तर्गत जो प्रावधान दिए गए हैं, उनके कार्यान्वयन के लिए हम सब प्रतिबद्ध हैं और सचिव, नराकास, जम्मू डॉ. अमर सिंह, सेवानिवृत्त हो गए हैं और अगली सचिव, नराकास, जम्मू डॉ. रमा शर्मा को कार्यभार सौंपा गया है।

संस्थान के मुख्य वैज्ञानिक डॉ. सुरेश चन्द्र ने बैठक में सभी कार्यालय प्रमुखों एवं उपस्थित गणमान्य व्यक्तियों का अपने संस्थान की ओर से व नगर राजभाषा कार्यान्वयन समिति के मंच से उपस्थित अधिकारियों का स्वागत करते हुए अपने अध्यक्षीय संबोधन में कहा कि भारत सरकार के अधीन यह समिति केन्द्रीय कार्यालय/बैंकों/उपक्रमों का एक संयुक्त मंच है इस मंच के माध्यम से हिन्दी कार्यान्वयन एवं अपनी समस्याओं के निदान हेतु छः महीने के अन्तराल पर हम सबको पुनः मिलने का अवसर मिलता है। चूंकि हिन्दी कार्यान्वयन प्रत्येक कार्यालय में कार्यालय कार्य का हिस्सा है। उन्होंने अपने विचार व्यक्त करते हुए अन्य प्रदेशों के साथ हिन्दी प्रचार-प्रसार की तुलना करते हुए कहा कि प्रत्येक राज्य स्तर पर अलग-अलग अनुपात में हर प्रदेश में हिन्दी का प्रयोग हो रहा है। उन्होंने अपनी प्रतिक्रिया व्यक्त करते हुए बताया कि व्यक्ति विशेष द्वारा चर्चा के दौरान सबसे अधिक वार्तालाप का माध्यम हिन्दी ही है। हम सबको अपने कार्यालय स्तर पर हिन्दी प्रयोग बढ़ाना है। उन्होंने बैठक में उपस्थित होने के लिए सबका हृदय से आभार व्यक्त किया। अन्त में राजभाषा कार्य के श्रेष्ठ निष्पादन हेतु विभिन्न कार्यालयों को शील्ड एवं प्रमाण पत्र भी वितरित किए गए।

धन्यवाद प्रस्ताव संस्थान के नियंत्रक प्रशासन, श्री पंकज बहादुर ने बैठक में अध्यक्ष महोदय एवं उपस्थित नराकास जम्मू के सभी केन्द्रीय कार्यालयों/बैंकों/उपक्रमों के कार्यालय प्रमुखों एवं नगर के प्रिन्ट व इलैक्ट्रानिक मीडिया के सभी संवाददाताओं का आभार व्यक्त किया।

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