CONTENTS

CONTENTS

DIRECTORS REPORT

I take pleasure in presenting this Annual Report of CSIR- Indian Institute of Integrative Medicine, Jammu which covers the highlights of the work carried during the year 2011-12. This year particularly remained very hectic where in several brainstorming sessions were held within the institute and in the presence of several invited subject matters experts to identify the programms and specific projects that institute focuss would during 12th Five Year Plan period.

Several dignitaries visited the institute this year, prominent among themwas the visit of the Director General CSIR and Secretary to Government of India, Prof. S. K. Brahmachari who met Hon'ble Chief Minister of Jammu and Kashmir, Mr Omar Abdullah and the members of the Pharmaceutical Industry in Jammu region in connection with the programms on employment and revenue generation in the state. Hon'ble Chief Minister of Jammu and Kashmir, Mr Omar Abdullah also visted Institute both at Jammu and Srinagar and appreciated the work being pursued at IIIM. He also released the book entitled "*Harnessing buiodiversity and biotechnology for progress of Jammu and Kashmir State*" during the Scientific Advisory committee held in IIIM. Mr Omar Abdullah also laid the foundation stone of a modern GLP animal house in the campus of IIIM, Jammu.

Hon'ble Parliamentary committee for the promotion of Hindi led by Sh Satyavrat Chaturvedi, Member Parliament visited the institute to have the assessment of the work being done by various central Government offices banks etc in Jammu region for the promotion of Hindi. The Hon'ble committee expressed it satisfaction on the efforts being made by IIIM to promote Hindi as official language.

During this year the institute also released a new strain of hyper-productive*Monrada citradora* Cerv. ex. Lag – A rich source of thymoldesignated as IIIM (J)MC-02 by following mass selection technique,. The major chemical constituent of this strain is thymol which ranges from 70-85 % and has pleasant *Trachyspermum ammi* (ajwain) like fragrance.

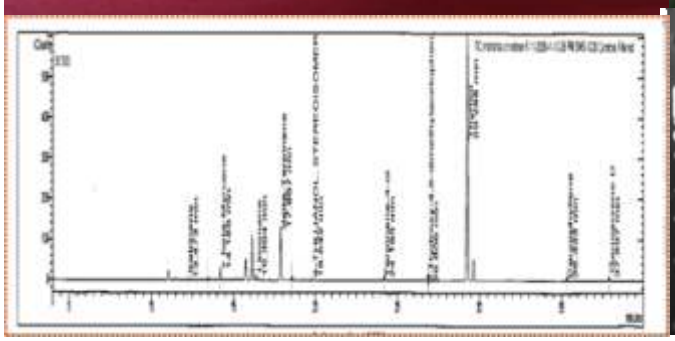
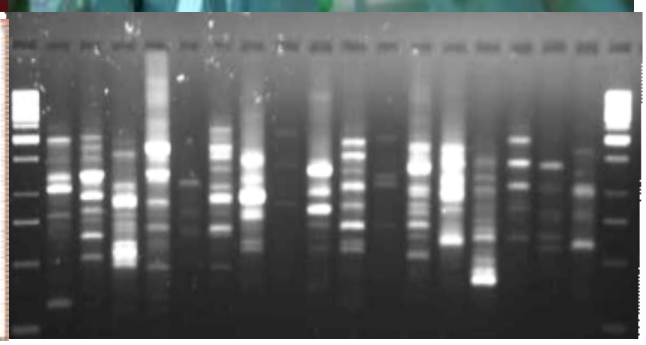
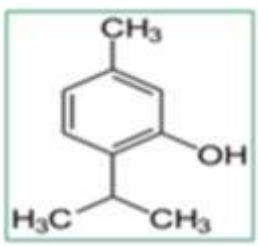
The following details in this report shall present to you the flavour of scientific work being carried out in the institute.

(Ram Vishwakarma)

1. INDIAN INSTITUTE OF INTEGRATIVE MEDICINE, JAMMU RELEASES *MONARDA CITRIODORA* CERV. EX LAG- A RICH SOURCE OF THYMOL





Introduction

Monarda citriodora Cerv.ex Lag.commonly known as Thymo-Herb belonging to family lamiaceae, is an important aromatic herb and a natural source of essential oil. The genus name is after Nicholas Monardes, a Spanish physician from Seville who wrote in the 16th century about New World Medicinal plants. The species name comes from Latin for "Citrus smell". It occurs on limestone glades, rocky prairies in Missouri and ranges from Texas and Mexico north to Missouri and Kansus. Therefore the plants prefers light (sandy), medium (loamy) and heavy (clay) soils. It requires moist soil. The plant was introduced to judge its performance in terms of essential oil production and its quality characteristics under sub-tropical environment.

1.0 Plant Description

It attains a height of 50-90 cms. Leaves are 3 to 8 cm long, soft textured, lance shaped ending in a point and have a very strong and pleasant Trachyspermum ammi (ajwain) like fragrance when crushed. Flowers are pink in color.

2.0 Improved Variety

Following mass selection technique, a hyper-productive strain of *Monarda Citriodora* Cerv. ex. Lag was developed and named as Thymo-Herb and designated as IIIM (J) MC-02. The major chemical constituent of this strain is thymol ranging from 70-85 % in its essential oil and have pleasant *Trachyspermum ammi* (ajwain) like fragrance.

3.0 Tissue Culture

Multiplication of 'elite' clone [IIIM(J)MC-02] of *Monarda citriodora* Cerv. ex Lag. By tissue Culture strategy: *In vitro* multiplication protocol was developed for elite selected clone [IIIM(J)MC-02] of *Monarda Citriodora* Cerv.ex. Lag. Explants collected from field grown plants growing at Experimental Farm, Chatha were inoculated onto MS media with different combination of PGRs. Proliferative high frequency axillary shoot initiation was observed on treatment containing lBA and BAP (1 mg/1). Regenerated shoots uniform in size were transferred to treatment lBA and Kn (1 mg/1) resulted 80% rooting. Plantlets were hardened and acclimatized under green house conditions with 80% survival rate. RAPD and ISSR marker based analysis of in vitro plants confirmed genetic uniformity of Micro propagated plants.

5.0 Therapeutic and General Uses Edible Use

Edible part of the plant is leaves-raw or cooked. They are used as flavouring and garnishing agent in salads, summer punches and as condiment in cooked foods and also used to make a refreshing lemon tea. It makes a lovely aromatic tea; its flowers are edible.

Medicinal Uses

Uses in colds, coughs, fevers, flue, bronchial problems, colic, flatulence, nausea, stomach cramps, menstrual irregularity, bowel ailments, to expel worms, induce sweats, headaches etc., most often as a tea especially for headaches; other medicinal treatment methods for bronchial problem include inhalation of the extracted essential oil or vapour therapy. The essential oil used as an insect repellant and in perfumery.

6.0 Agro-technology

6.1 Soil and climate

Monarda citriodora Cerv.ex Lag.can be grown on moderately fertile and well trained loam to sandy loam soils having good water holding capacity. Water-logged conditions should be avoided. Being a Rabi crop it has been trans-planted in November-December having 20-25°C ambient temperature.

6.2 Seed sowing

About 200-300 g seeds are enough to raise seedlings for planting one hectare of land. The seeds can be sown in nursery beds during first week of October and then transplanted during 3rd week of November or 1st week of December. It takes about 10-12 days for the seeds to germinate.

6.3 Land preparation

The land should be brought to fine tilth and laid out into plots of onvenient size depending upon the source of irrigation. It is desirable to add 10-15 tonnes of farm yard manure per hectare during the preparation of land and mixed properly in the soil.

6.4 Transplanting

It is recommended to plant the seedlings at 30x30 cm line to line and plant to plant to get the high herbage and oil yield per hectare. The plants are irrigated immediately after transplanting. During this operation, gap filling and replacement of the poor plants is done so that uniformity of the crop is maintained.

6.5 Manures and fertilizers

The crop responds well to the application of manures and fertilizers. The optimum fertilizer dose recommended for this crop is 100 kg nitrogen (as Urea) and 50 kg muriate of potash (K_2O) and 50 Kg single superphosphate (P_2O_5). Nitrogen is applied in three/four equal split doses.

6.6 Irrigation

Frequency of irrigation depends upon the moisture status of the soil and weather conditions prevailing during the crop season. Crop requires maximum irrigation 8-10 times for the successful crop production.

6.7 Weeding

First weeding is done one month after planting and second 4 weeks after the first. One hoeing in two months after transplanting is sufficient.

6.8 Diseases and Pests

No major attack of pests and diseases was observed.

6.9 Harvesting and yield

Harvest should be done in the

month of April-May at full blooming stage. Rainy periods should be avoided for harvesting which resulted in low essential oil recovery as well as poor quality of oil. It is estimated that on an average 25-30 tonnes per hectare per year herbage was obtained. On an average, 100-125 kg of essential oil obtained per hectare.

7.0 Distillation

The essential oil is obtained by steam distillation. Field distillation unit is also used for extraction of oil. It takes 3.0-4.0 hours for completing the process while steam operated system takes 2.0-3.0 hours only. Field distillation units are appropriate for small/medium farmers in view of their low cost.

8.0 Storage

Essential oil undergoes considerable changes in its chemical composition during storage. On longer period of storage, polymerization takes place resulting in decrease of thymol content, thus adversely affecting the oil quality. To avoid this type of loss, the essential oil should be completely dried by using anhydrous Sodium sulphate or any other dehydrating material before filling in the containers. The containers should be air-tight and filled to the brim, and preferably stored in dry, cold and dark condition.

9.0 Major chemical constituents/Chemical markers

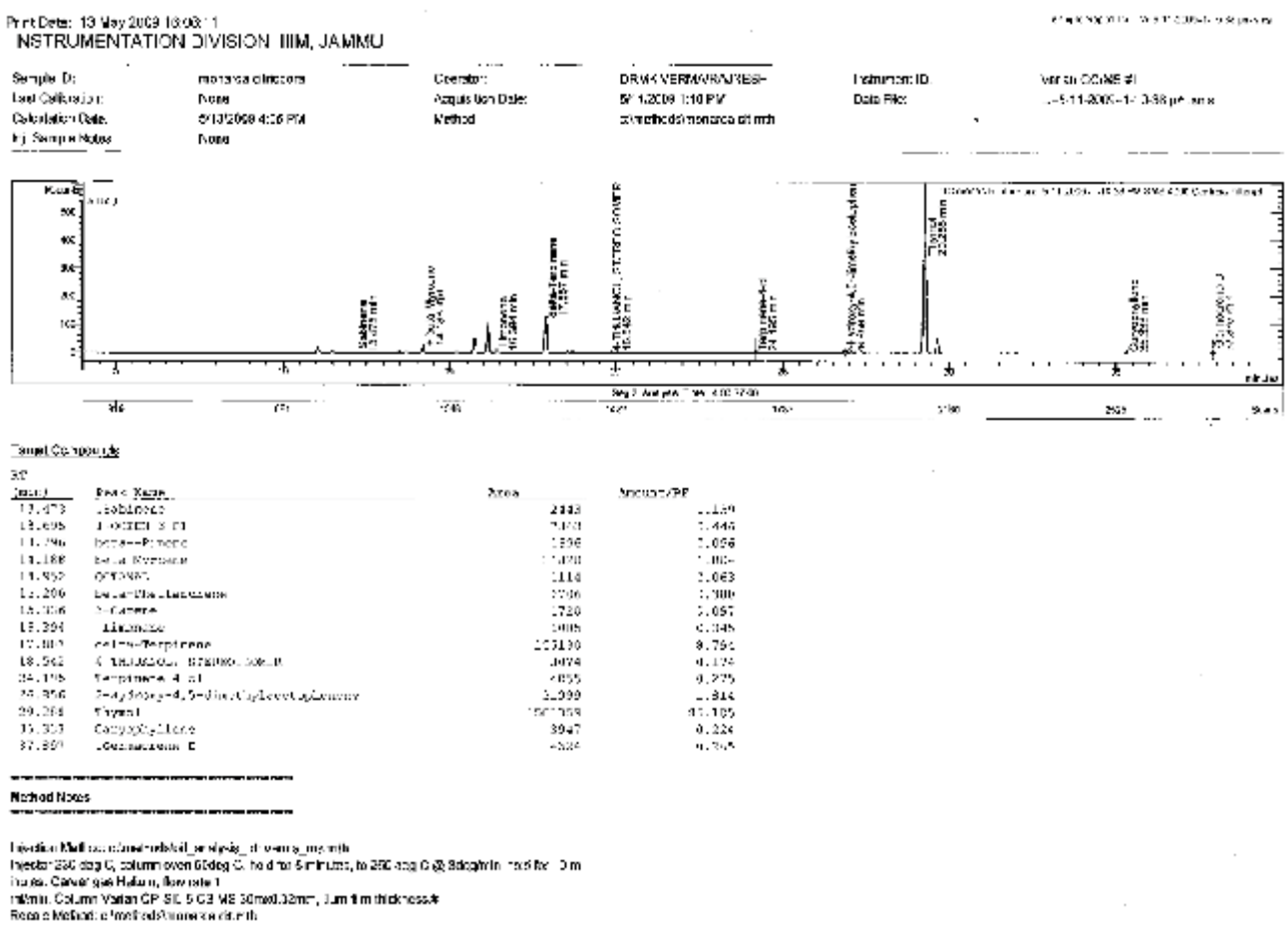
The essential oil consists of about eighteen chemical compounds identified and quantitated by GC/MS, out of which thymol (85 %) is the major chemical constituent (Table 1). The essential oil of this strain can be a better source of thymol.

Table 1: Physico-Chemical Profiling and GC-MS of *Monarda citriodora* Cerv. ex Lag. IIIM(J) MC-02:

[A]Physical Properties:-

Refractive Index $n_D^{25°C}$	:	1.49995
Specific Gravity $d^{25°C}$	:	0.927678
Thymol content	:	65-85 % (GC-MS)

[B] Chemical Profiling and GC-MS:-



10.0 Value Addition

Essential oil ex. *Monarda citriodora* Cerv.ex Lag., produced from Indian cultivar is an unique combination of thymol (major), carvacrol, thymol methyl ether and carvacrol methyl ether in addition to about 45 or so other major and minor compounds. Due to the presence of these compounds the essential oil has milder and soothing flavor when compared with oil of Ajwain (*Trachspermum copticum*) and can be used as better substitute.

Oil can also be utilized for the production of natural thymol with finer aroma (due to accompanied trace materials of natural origin), which is much in demand.

The oil remaining after separation of thymol followed by rectification to remove some undesirable constituents can be utilized as very fine perfumery/ flavouring material to impart spicy note to large number of high end preparations and may be compatible with water soluble materials.

11.0 Molecular profiling

Monarda citridora [IIIM(J)MC-02] was characterized by various ISSR and RAPD primers. With different primers, DNA showed unique profile depicting unique identity of *Monarda citridora*.Size of banding pattern was ranged between 250 bp - 3 Kb. Accessing the genetic fidelity of plants is also of utmost importance. Tissue cultured raised plants as well as field grown various plants were screened which showed uniform molecular profile suggesting the uniformity in the material.

12.0 Anti-cancer potential of essential oil

To evaluate the cytotoxic potential of essential oil of *Monarda citriodora*, it has been tested for the MTT cell viability assay in humanleukemia HL-60 cells. Essential oil of *Monarda citriodora* has significant cytotoxic potential in HL-60 cells, with IC_{50} value (concentration of test compound, which is required to inhibit 50% cell viability) of 22µg/ml. These results showed that essential oil of *Monarda citriodora*has significantanticancer potential in HL-60 cells.

13.0 Economics

Crop duration: Six Months

	Parameter (s)	Values (Range)
(a)	Fresh Herb Yield (Tonnes/ha)	25-30
(b)	Essential Oil Yield (kg/ha)	100-125
(c)	Cost of Production (Rs. /ha)	40,000-45,000
(d)	Value of Essential Oil (@ Rs 1000/kg)	1, 00,000 – 1, 25,000
(e)	Net profit (Rs./ha)	60,000- 80,000

14.0 Marketing

There is a great demand of thymol containing essential oils and it is increasing every year. Essential oil of *Monarda citriodora* has been accepted by pharmaceutical houses as an additional and alternative source of thymol. The prevailing price of the oil in Indian market is Rs. 1000/- per kg.

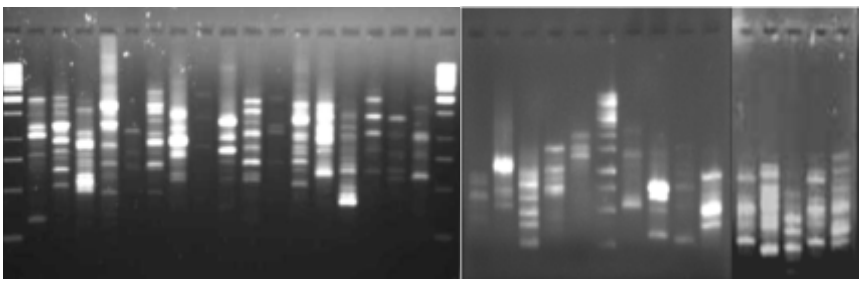


Fig 1: DNA fingerprint of *Monadracitridora* [IIIM(J) MC-02] using 32 different RAPD primers.(lanes 2-18, 20-35, left to right; lanes 1,19 are DNA ladders)

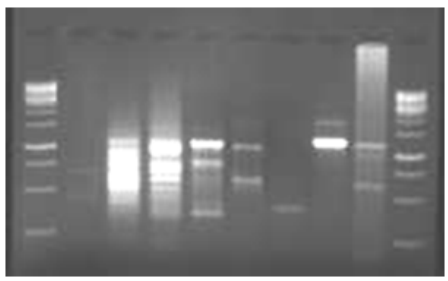


Fig 2: DNA fingerprint of *Monadracitridora* [IIIM(J) MC-02] using 8 different ISSR primers.(lanes 2-9, left to right; Extreme left and right are DNA ladders).

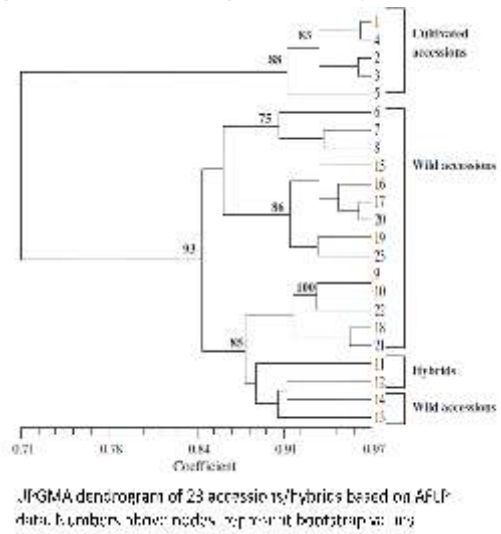
2. BIODIVERSITY AND APPLIED BOTONY

2.1 Status of new species, W. ashwagandha, to the cultivated taxon of Withania somnifera

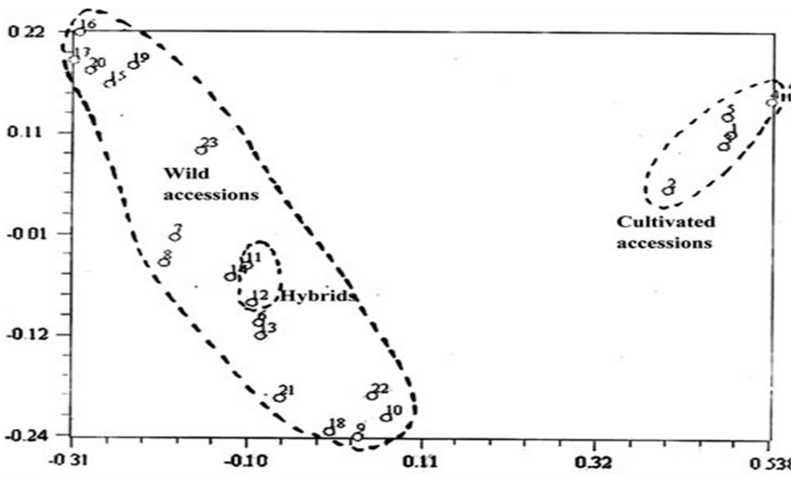
Arun Kumar, Bilal A. Mir and Sushma Koul,

Realizing the inconsistencies that exist in the extent and nature of differentiation in the *Withania somnifera* genetic resources in India, the 21 cultivated and wild accessions, and the two hybrids were investigated for morphological, cytogenetical, chemical profiling, and crossability features. Results from multidisciplinary approaches, especially that of the DNA nucleotide sequencing and DNA marker fingerprinting, and crossability assays reveal great discrete genomic diversity between wild and cultivated *W. somnifera*to warrant distinct species status, namely *W. ashwagandha,*for cultivated and wild taxa of *W. somnifera*. Chloroplast DNA diversity and somatic chromosome number (2n = 48) were not helpful in identifying the differences but other approaches, especially restriction endonuclease digests, types and sequence length composition of ITS 1 and ITS 2 of nuclear ribosomal DNA, AFLP fingerprinting, and crossability barriers unambiguously provided startling discrete differences between the cultivated and wild accessions, indicating a clear division of *W. somnifera*into two distinct lineages. Because of the unique characteristics of its nuclear genome, and strong crossability barriers vis-a`-vis wild accessions of *W. somnifera*, the cultivated accessions should be relegated to the rank of the separate species, *W. ashwagandha*.

a–f Morphological features of the wild (a–c) and cultivated (d–f) accessions *of Withaniasomnifera*

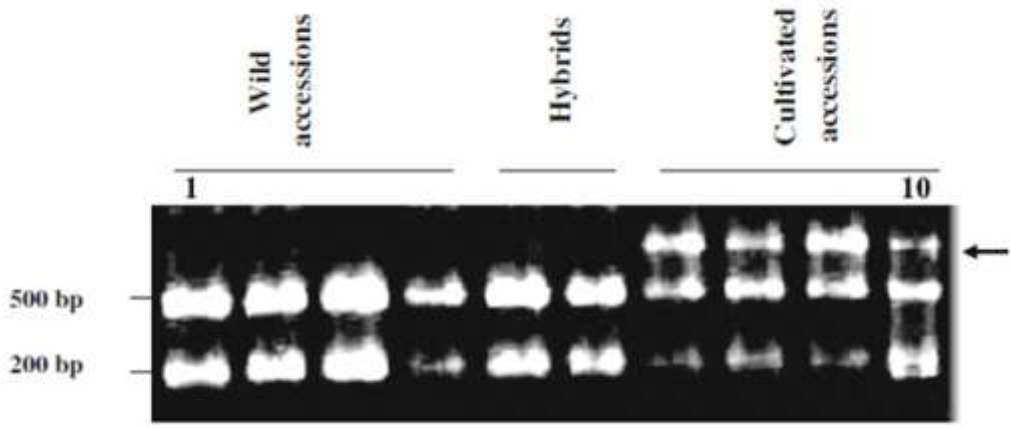


UPGMA dendrogram of 23 accessions/hybrids based on AFLP data. Numbers above nodes represent bootstrap values



Principal coordinate analysis (PCO) plot of 23 accessions/hybrids

Substitution and indel (insertion/deletion) events in ITS sequence of 23 accessions/hybrids.Solid circles represent single-point mutations. Short vertical bars indicate indels



Representative restriction fragment size patterns of amplified ITS1-5.8S-ITS2 region in ten accessions /hybrids by EcoRV.The arrow represents the unrestricted 600-bp band in cultivated accessions

2.2 Metabolic shift from secondary metabolite production to induction of anti-oxidative enzymes during NaCl stress in *Swertia chirata* Buch. - Ham.

Esha Abrol, Dhiraj Vyas and Sushma Koul

A study was envisaged to understand *in vitro* induction of marker secondary metabolites using NaCl elicitation. 50 and 100 mM of NaCl concentrations were applied on 1-monthold static shoots culture of *Swertia chirata* Buch.-Ham., an endangered medicinal plant having high medicinal value. Plants were assessed for cellular damage, anti-oxidative enzymatic system and production of secondary metabolites. There was significant (p B 0.05) increase in secondary metabolites at 50 mM NaCl without any cellular damage or induction of anti-oxidative enzymes. Initial increase in metabolic content of secondary metabolites was observed during 100 mM NaCl treatment, which falls back to normal levels at the seventh day. There wasconcurrent induction of scavenging enzymes during this period. Results suggest channelling of different defence strategies in response to differential NaCl treatment. Biochemical relationship between induction of anti-oxidative enzymes and production of secondary metabolites has further been discussed in light of physiological requirements. In conclusion, results showed that 50 mM NaCl is sufficient to invoke elicitation for induction of secondary metabolites in *S. chirata*, which is desirable for *in vitro* production of secondary metabolites. While induction of enzymatic defense is desirable for normal plant growth under physiological and biochemical stress, it would impede the secondary metabolite productionOur results suggest that there is preferential channeling between two main defence processes. Under low NaCl concentration, plants divert their metabolism towards production of secondary metabolites, which are believed to be more energy consuming. Production of secondary metabolites has been a complex metabolic process involving a large number of genes thereby, taking a large chunk of metabolic energy Further, at 100 mM NaCl concentration, efforts were made to maintain metabolic homeostasis by increasing expression of anti-oxidative enzymes than secondary metabolites. Present investigation suggested that production of secondary metabolites in S. chirata is the initial response for defence during 50 mM NaCl. There was no significant cellular damage and induction of enzymatic defence during this time. This allow plants to divert their metabolism towards costly process of secondary metabolite production. Metabolic shift from growth to defence is evident from the stunted growth.During high NaCl concentration (100 mM), cellular damage increases along with the increase in ROS. This shift the

metabolic tilt of plants towards increased production of anti-oxidative enzymes. Metabolic content of marker glycosides decreased significantly (p B 0.05) during this time as is evident from Table 1. Theory of optimal defence proposes allocation of resources based on the risk of attack and the value of particular tissue To the best of our knowledge, this is the first report of preferential channelling of metabolites between induction of enzymatic

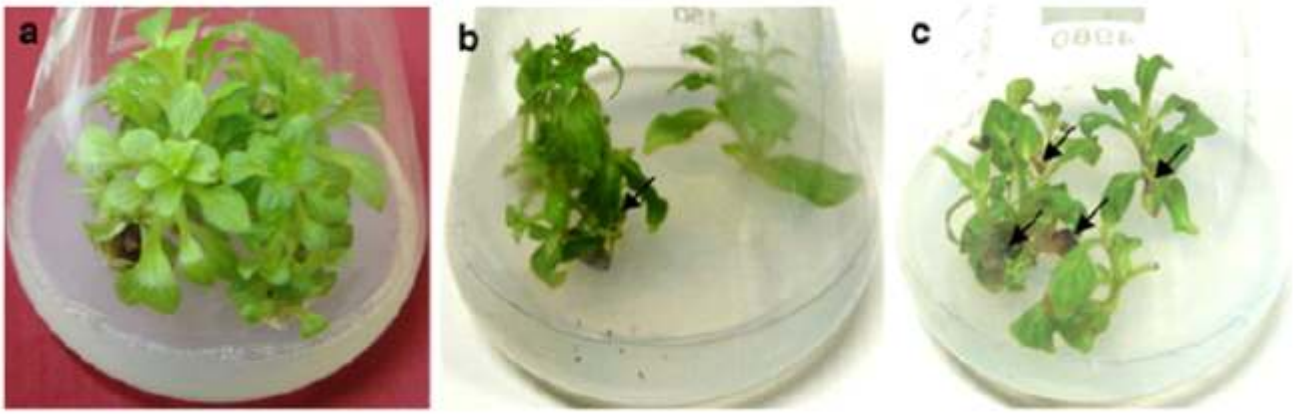


Fig 1.Shoot cultures of *Swertia chirata* as seen after 7 days of 0 mM (a), 50 mM (b), and 100 mM (c) salt treatment. Marked areas represent necrotic regions after 7 days

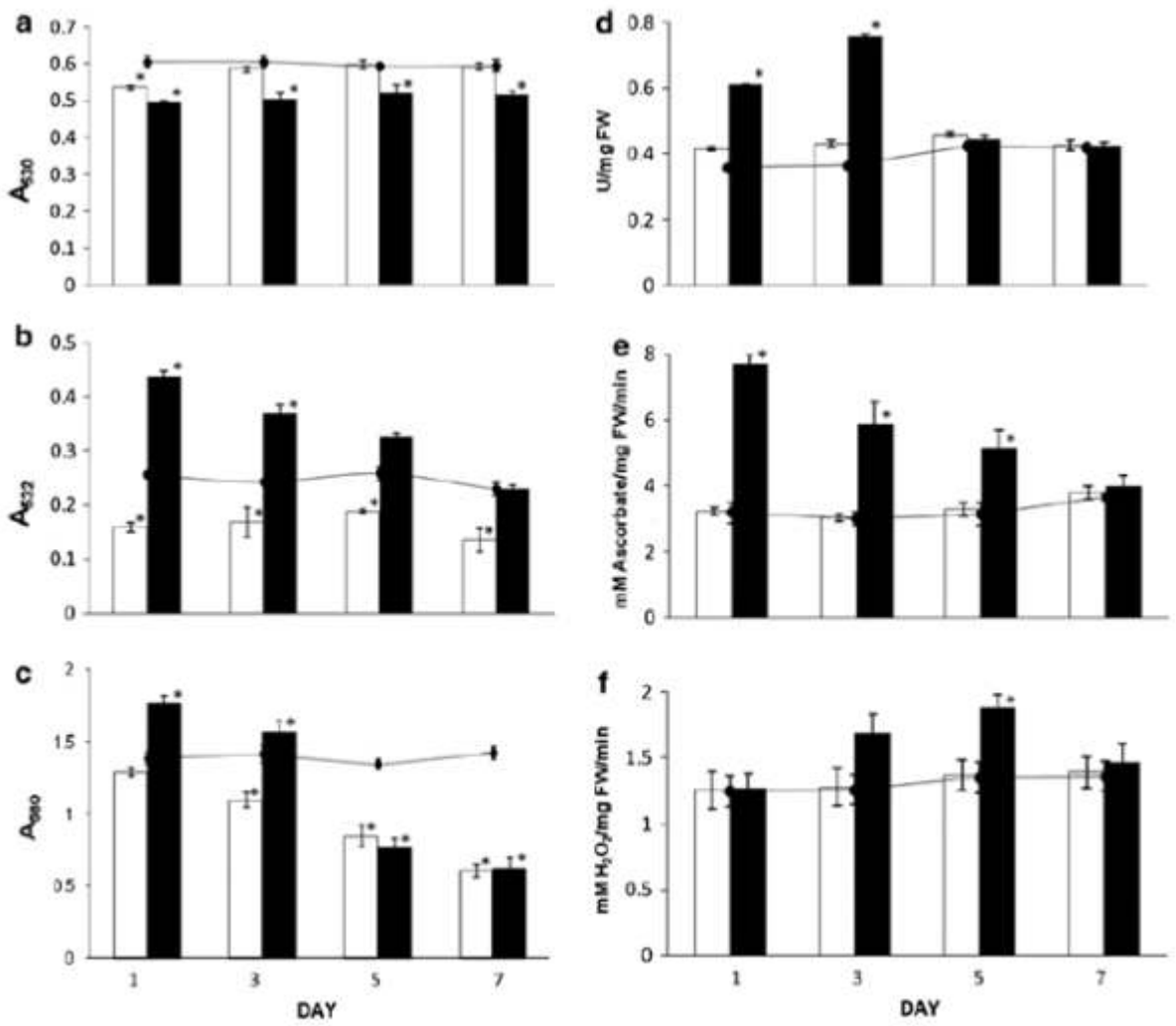


Fig. 2 Metabolic changes in TTC reduction (**a**), lipid peroxidation (**b**), superoxide anion content (**c**), SOD activity (**d**), APX activity (**e**), and CAT activity (**f**) in *Swertia chirata*. Biochemical activities were measured following 0 mM (*filled circles*), 50 mM (*open bars*), and 100 mM (*filled bars*) NaCl concentration in 1-month-old shoot cultures. Values are mean of three independent replicates ± SD. Significantly different values at $p \leq 0.05$ are represented by *

2.3 Podophyllum lignans array of Podophyllum hexandrum Royle populations from semi-desert alpine region of Zanskar valley in Himalayas

S. Kitchlu, G. Ram, Sushma Koul, Kiran Koul, K.K. Gupta and Ashok Ahuja

Podophyllum hexandrum Royle (Syn. *P. emodi* Wale) a perennial rhizomatous herb found in alpine region distributed in the entire range of Himalayas from Ladakh to Sikkim at an altitude of 3000-4200 m asl. is an preferred commercial source of podophyllum lignans. It contains three times more Podophyllotoxin than the American species, *P. peltatum*. The present study was aimed to investigate variation of podophyllum lignans contents based on six marker compounds viz. Podophyllotoxin; Deoxypodophyllotoxin; Picropodophyllotoxin; Podophyllotoxin ß-D glucopyanoside; Isopicropodophyllone; 4' Demethyldeoxypodophyllotoxin, ß-D-lucopyanoside, in *P. hexandrum* population growing at three locations. Further, ontogenetic and morphogenetic variations of Podophyllum lignan contents were studied to investigate dynamics of accumulation of these compounds. Representative collections from three locations viz., Panikhar, Padam and Tangoli located in Trans Himalayan semi-desert region of Zanskar valley were harvested at three stages (dormancy, active growth and maturity). Plants were dissected into root, rhizome and rhizome-buds, dried separately and assayed for Podophyllum lignan contents by high performance liquid chromatography.

The present study which is a first report of its kind where rhizome buds have shown rich accumulations of Podophyllum lignans offer a logical alternative approach to utilize plant parts (rhizome-buds) as renewable source other than the rhizomes to extract podophyllotoxin. This is likely to make cultivation of the species feasible besides sustainable conservation strategy of (*Podophyllum hexandurm*) Himalayan May-Apple by restricting unwise harvesting of the rhizome.

2.4 PODOPHYLLUM HEXANDRUM" – A Versatile Medicinal Plant

Parvaiz H Qazi, Abila Rashid and Sami A Shawl

Podophyllum hexandrum a perennial herb, bearing the common name Himalayan Mayapple known as *Aindri* (a divine drug) in ancient times is native to the lower elevations in and surrounding the Himalaya. It has been reported to be used through the ages and in modern times as an intestinal purgative and emetic, salve for infected and necrotic wounds, and inhibitor of tumor growth. The rhizome of the plant contains a resin, known generally and commercially as Indian Podophyllum Resin, which can be processed to extract podophyllotoxin or podophyllin, a neurotoxin. Podophyllotoxin is the major lignan present in the resin and is a dimerized product of the intermediates of the phenylpropanoid pathway. The starting material of etoposide (Vepeside), an FDA approved anticancer drug is podophyllotoxin and has been used to treat testicular cancer as well as lung cancer by inhibiting replication of cancer cells. Podophyllotoxin finds use as a precursor for the semi-synthetic topoisomerase inhibitors in the treatment of leukemias, lung and testicular cancers, dermatological disorders like warts, rheumatoid arthritis and psoriasis. It also has numerous applications in modern medicine by virtue of its free radical scavenging capacity. An extract of *P. hexandrum* has been shown to provide approximately 80% whole-body radioprotection in mice. Total synthesis of podophyllotoxin is an expensive process and availability of the compound from natural resources is an important issue for pharmaceutical companies that manufacture these drugs. The Indian *P. hexandrum* is superior to its American counterpart, *P. peltatum* in terms of its higher podophyllotoxin content (>5%) in dried roots in comparison to only 0.25% of *P. peltatum*. Thus, taking into consideration present status of *P. hexandrum* in general, it needs immediate attention for conservation,

Fig.1: Shows *Podophyllum hexandrum* growing wild in Kashmir – India

in depth studies for improving propagation techniques and podophyllotoxin production, encouraging its cultivation and a detailed study of its phytochemical diversity, particularly of its marker compounds like podophyllotoxin and its glycosides.

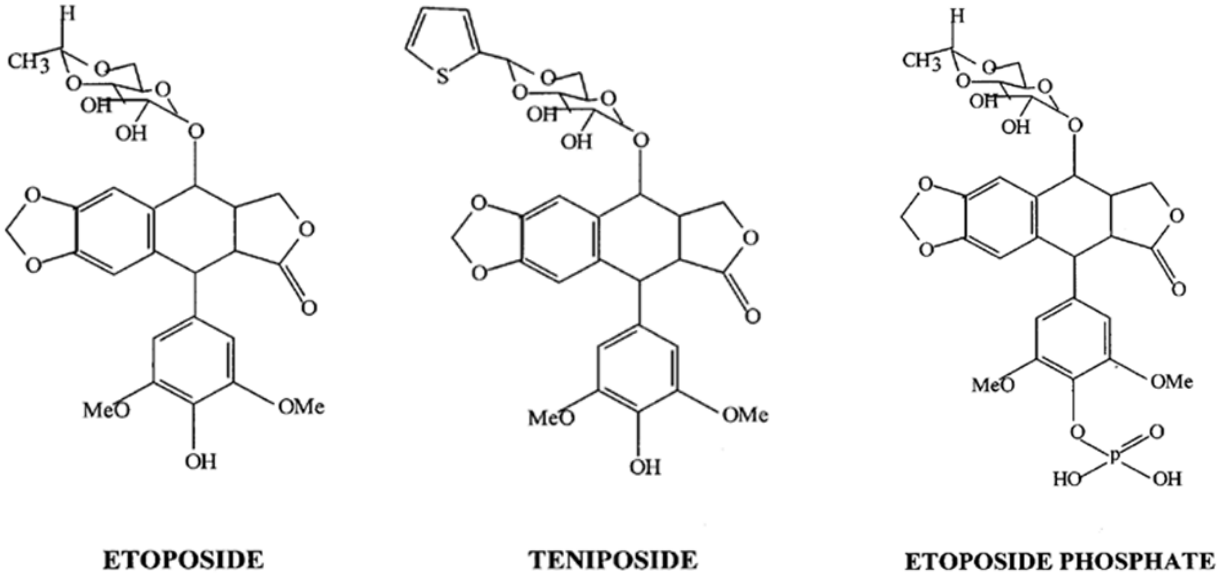


Fig 2: Etoposide Teniposide Etoposide phosphate

3. PLANT BIOTECHNOLOGY

3.1 Analysis of SSR dynamics in chloroplast genomes of Brassicaceae family

Praveen Awasthi, Sumit G. Gandhi and Yashbir S. Bedi

Simple Sequence Repeats (SSRs) or microsatellites are tandem repeats of mono-, di-, tri-, tetra-, etc. nucleotide motifs. SSRs are present abundantly in most eukaryotic genomes. SSRs affect several cellular processes like chromatin organization, regulation of gene activity, DNA repair, DNA recombination, etc. When present in protein coding DNA segments, their expansion or contraction can impact protein's function. Several human diseases have been linked to expansion of trinucleotide microsatellites within protein coding genes and have been dubbed trinucleotide repeat disorders. Through "guilt by association", several microsatellite loci in plants have been linked to stress tolerance, disease resistance, domestication events and various agronomic traits. In the present study we have probed various nucleotide repeat motifs (NRMs) / types of SSRs present in chloroplast genomes (cpDNA) of 12 species belonging to

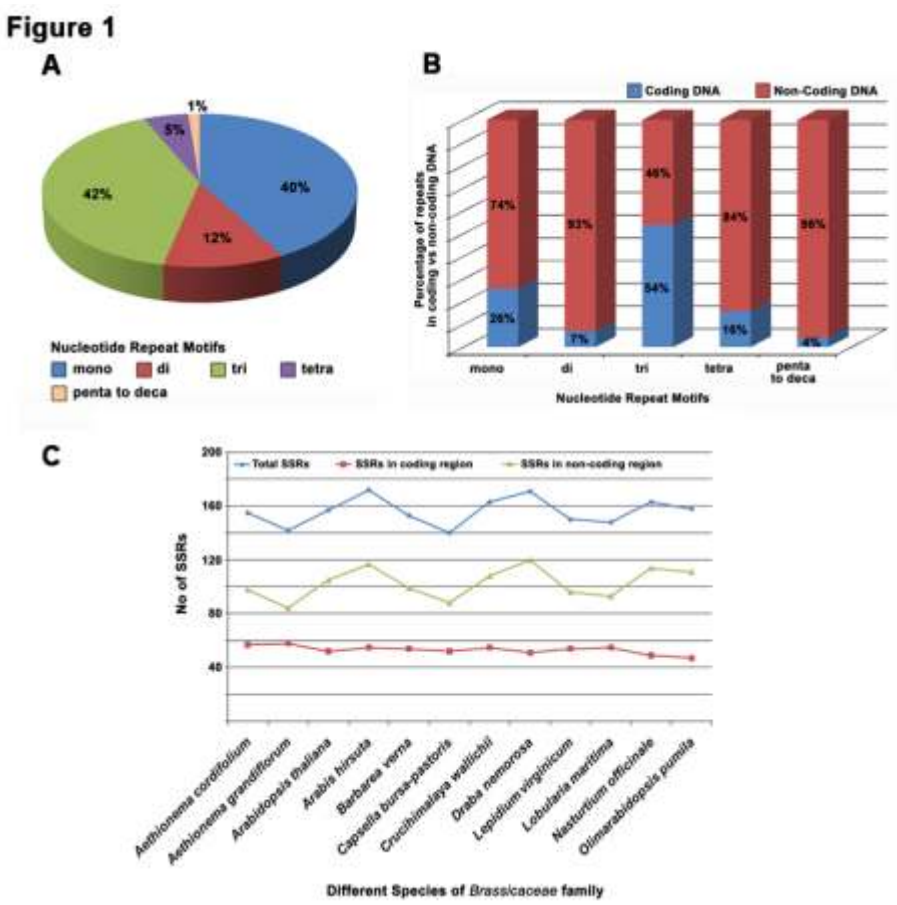


Figure 1.Distribution of Nucleotide Repeat Motifs (NRM) in Chloroplast genomes of 12 species belonging to Brassicaceae family.(a) Pie chart revealing the percentages of different types of NRMs (SSRs) in Brassicaceae (b) Bar graph indicating percentage of various NRMs in coding or non-coding regions of chloroplast DNA (c) Species wise distribution of total SSRs in coding or non-coding regions of chloroplast DNA. Note: Percentages have been rounded to nearest integer.

Brassicaceae family (Fig1 a). NRMs show a non-random distribution in coding and non-coding compartments of cpDNA. As expected, trinucleotide repeats are more common in coding regions while other repeat motifs are prominent in non-coding DNA (Fig 1 b). Total numbers of SSRs in coding region show little variation between species while considerable variation is exhibited by SSRs in non-coding regions (Fig 1 c). Finally, we have designed universal primers that yield polymorphic amplicons from all 12 species (Fig 2 a,b). Our analysis also suggested that amplicon length polymorphism may not always show significant relationship with sequence polymorphism of SSR amplicons in cpDNA of Brassicaceae family (Fig 2 c,d).

Figure 2

A

Universal Primers			
	Forward	Reverse	Tm
Primer Set 1	CTAGACGATGGGGGCATACT	GCCTTACCATGGCGTTACTC	59
Primer Set 2	CCCTCGGTAACGTGACATAA	GCATTTGTAATGCGATGGTC	58

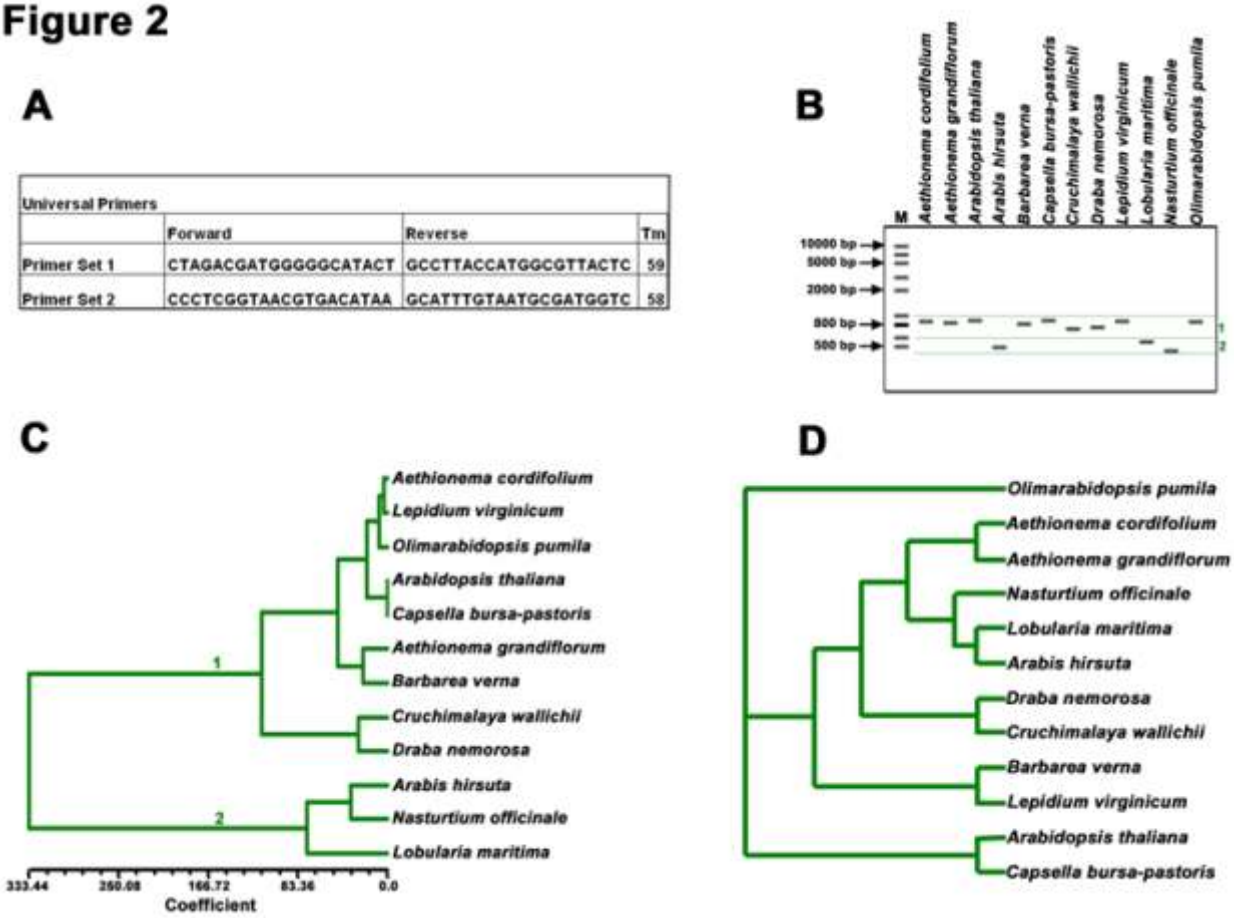


Figure 2.Microsatellite polymorphism in Chloroplast genomes of 12 Brassicaceae species. (a) Universal primer sets exhibiting amplicon length polymorphism (b) Simulated DNA electrophoretogram of predicted amplicons using universal primer set 1 (c) Phylogenetic tree based on amplicon length polymorphism using universal primer set 1 (d) Phylogenetic tree based on amplicon sequence polymorphism using universal primer set 1

3.2 Development of Chloroplast Microsatellite Markers for Phylogenetic analysis in Brassicaceae

Praveen Awasthi, Irshad Ahmad, Sumit G. Gandhi and Yashbir S. Bedi

Brassicaceae, commonly referred as the 'mustard family' or 'cabbage family', generally consists of herbaceous plants with annual, biennial or perennial lifespans. The family is both economically as well as scientifically important as it includes major crop species like *Brassica napus* and model organism like *Arabidopsis thaliana*. However, Brassicaceae is also known for problems with respect to the phylogenetic relationships amongst the taxa within the family.

Microsatellites infest the genomes of most eukaryotic species and often exhibit length polymorphism. Relative conservation of the flanking regions, allow the variable length microsatellites to be used as locus specific, co-dominant, genetic markers across taxa. Microsatellite analysis is often used for constructing phylogenies, in both plant and animal systems. Though considerable data exists on using nuclear microsatellites to infer phylogenetic relationships, the potential of chloroplast microsatellites (cpSSR), in this regard, remains largely unexplored. Here, employing *in silico* tools, we have explored the possibility of using chloroplast microsatellites for plotting

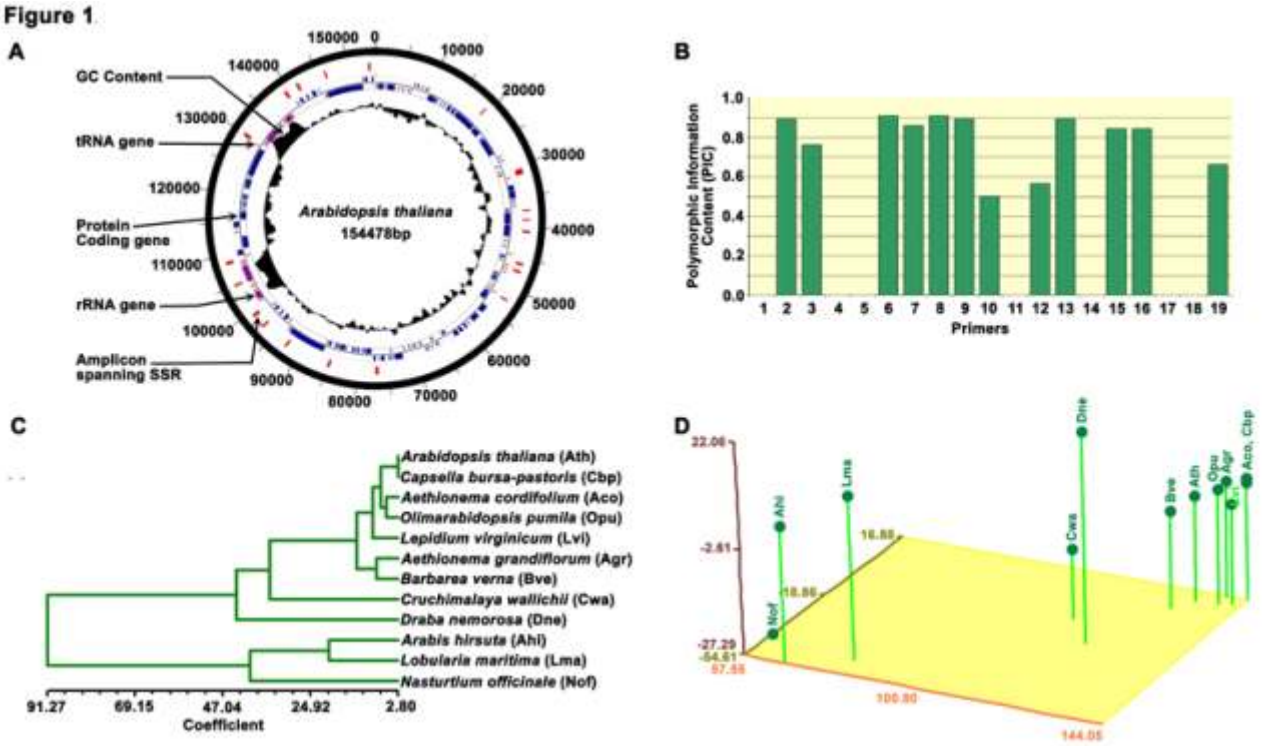


Figure 1.Phylogenetic analysis of Brassicaceae using Universal Primers spanning chloroplast Microsatellites

(a) Circular map of cpDNA of *Arabidopsis thaliana* depicting the locations of amplicons resulting from universal primers, the rRNA, tRNA and protein coding genes as well as GC content variation throughout the cpDNA. (b) Bar graph showing the Polymorphic information content (PIC) values of amplicons resulting from 19 universal primers (c) Phylogenetic tree resulting from UPGMA clustering of SSR amplicon length polymorphism data (d) Principal Component analysis of SSR amplicon length polymorphism data using the variance-covariance method.

phylogenetic relationships within mustard family. Microsatellite repeats were scanned in 12 chloroplast genomes (cpDNA) of Brassicaceae, regions flanking these repeats were aligned and 19 universal primer pairs were designed. 15 of these primer pairs yield polymorphic amplicons, that are more or less evenly distributed through the chloroplast genomes. The polymorphic information content (PIC) of these primers ranges from 0.5 to 0.91 and Expected Heterozygosity (Het) ranges from 0.53 to 0.92. Finally amplicon length polymorphism data was analyzed by principal component analysis (PCA) and clustered using UPGMA algorithm to plot phylogenetic tree based on cpDNA microsatellite variation (Figure 1). Finally, using PCR, we have validated three primer pairs on a limited 'test set' of plants, different from those used in computational analysis (Figure 2).

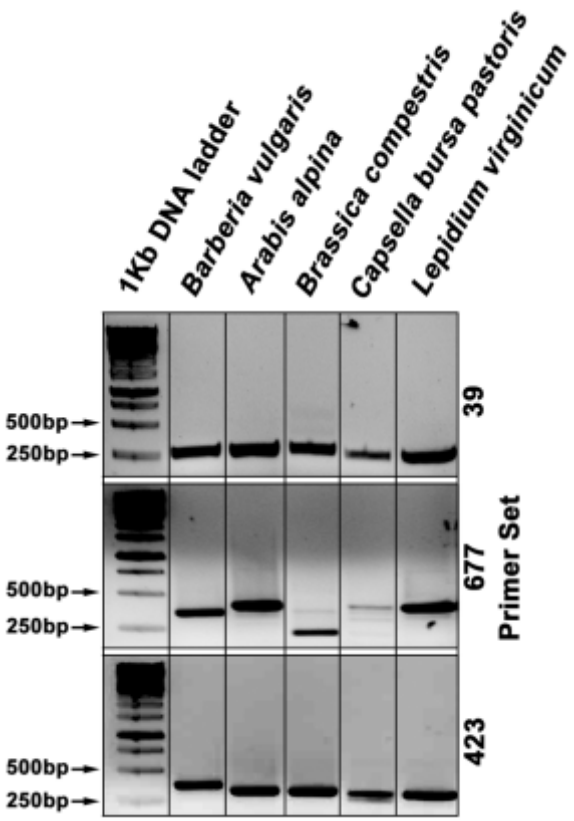


Figure 2. Experimental validation of Universal Primers designed for phylogenetic analysis of Brassicaceae

Agarose gels depicting PCR amplicons using three universal SSR primers on a limited 'test set' of plants, different from those used in computational analysis

3.3 Development of EST-SSR markers for *Zingiber officinale* Rosc. with transferability to other species of Zingiberaceae.

Praveen Awasthi, Sumit G. Gandhi, Pankaj Pandotra, Suphla Gupta and Yashbir S. Bedi.*

The family Zingiberaceae (monocotyledon) includes several medicinally important genera including Zingiber. *Zingiber officinale* Rosc.commonly known as 'ginger' worldwide is extensively cultivated and used as a spice. It is also used in traditional systems of medicine for treatment of headache, nausea, colds, arthritis, rheumatic disorders and muscular discomfort. Though mostly propogated vegetatively, considerable diversity is observed in cultivated ginger genotypes for various morphological, physiological and agronomic traits. The present study aims to develop markers for assessment of diversity at genetic level.

Large-scale genomics has generated a huge number of expressed sequenced tags. A large number of these fragments are sequences that translate into protein coding genes. Sometimes tandem repeats of mono, di, tri or tetra nucleotide motifs, commonly known as simple sequence repeats (SSRs) may be present in these sequences. Such repeats are prone to mutations changes, caused by replication slippage, which may result into contraction or expansion of these nucleotide repeats. PCR primers designed in the conserved flanking sequences can be used to assess any change in the length of SSRs amongst various accessions.

Such markers termed EST-SSRs also exhibit high degree of cross species transferability. In present study, we have analyzed about 38,000 EST sequences for the presence of SSRs. In total 531 SSRs (-mono to –deca) were found. Out of these 130 were –mononucleotide repeats, 211 dinucleotide repeats and 65 trinucleotide repeats. Amongst trinucleotide, AUU was the most common repeating element. Out of the 531 loci, primers could be designed only for 348 loci. 25 primers pairs were randomly selected for validation in 25 accessions collected from various areas of northwestern Himalayas. 16 primers resulted in successful amplification from various accessions of *Zingiber officinale*, though variation with respect to size was rarely observed. Further cross species transferability of these markers was tested against other species of Zingiberaceae (Table 1).

Primer set:	Curcuma longa	Curcuma amada	Curcuma Rignoza	Curcuma aungsifolia	Curcuma caesia	Zingiber zerumbet	Zinger heidihium	Curcuma Aromatics
423	-	+	-	+	-	-	+	-
440	-	-	-	-	-	-	+	-
452	+	+	+	+	-	+	+	-
454	-	-	-	-	-	+	+	-
456	+	+	+	+	-	+	-	+
459	+	-	-	+	+	+	+	-
466	+	+	+	+	+	+	+	+
472	-	+	-	-	-	+	+	-
475	-	-	-	-	-	+	+	-
480	+	+	+	+	-	+	+	+
481	+	-	-	-	-	-	+	+
486	+	+	+	+	-	+	+	+
495	+	+	+	+	+	+	+	-
497	-	-	-	-	-	+	+	-
511	-	-	-	-	-	-	-	-
512	-	+	-	-	-	+	+	-

Table 1. Cross species transferability of EST-SSR markers developed for *Zingiber officinale*. + indicates presence of amplification while – indicates absence of amplification. Along with presence/absence of amplification, size variation was also observed in amplicons from different species.

3.4 Microsatellite DNA marker isolation from *Picrorhiza kurrooa* Royle ex Benth by magnetic capture

M. A. Hussain, R. K. Aggarwal, Yashbir S. Bedi and M. Z. Abdin

Microsatellites or Simple Sequence Repeats (SSR's) are codominant markers used to identify both the alleles of a gene in a heterozygous individual. Microsatellite markers isolated de novo from a species render them species specific and are used for single-locus genotyping of a species. Multi-locus approaches may be convenient but have some technical and/or analytical drawbacks, such as dominance (i.e. only one allele identified where there is no possibility to discriminate between homozygous and heterozygous individuals). As a consequence of simultaneous visualization of many marker alleles, multi-locus data are typically analyzed by pair wise comparison of complex banding patterns that only have meaning relative to others in the same study, thus results are to a limited extent comparable among studies. In contrast, single-locus markers are usually characterized by co-dominance (i.e. both alleles identified in heterozygous individuals) and thus are more flexible and supply more robust and comparable data. Since techniques detecting heterozygotes (i.e. co-dominant markers) and providing data on allelic differences are desirable, microsatellites (SSR's) are considered to be the most suitable markers. The abundance of microsatellites depends upon the density of these motifs in the genome, which varies from organism to organism. Microsatellite isolation by conventional non–enriched protocols based on colony hybridization for screening of a large number of colonies for microsatellite motifs is labour intensive and less effective due to the occurrence of artifacts or hybridization signals with false positives. To overcome this problem various strategies to increase the efficacy of mining microsatellites have been successfully applied. These strategies primarily employ magnetic capture of microsatellite motifs by streptavidin coated magnetic beads and mixed biotin labeled probes hybridization capture strategy. By this technique, microsatellite sequences (EU883601 to EU883622 and FJ617210 to FJ617223) were isolated from an endangered medicinal herb Picrorhiza kurrooa Royle ex Benth (Royle, 1835). Our study is the first report of the isolation of species specific microsatellite DNA markers from *Picrorhiza kurrooa*. Types of motifs and their frequency distribution is presented in Figures 1 & 2.

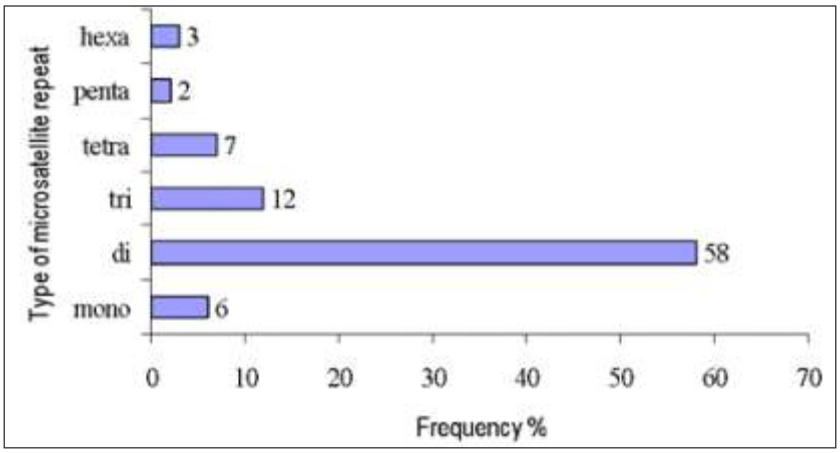


Figure 1.Frequency of the distribution of microsatellite repeat motifs in *Picrorhiza kurrooa.*

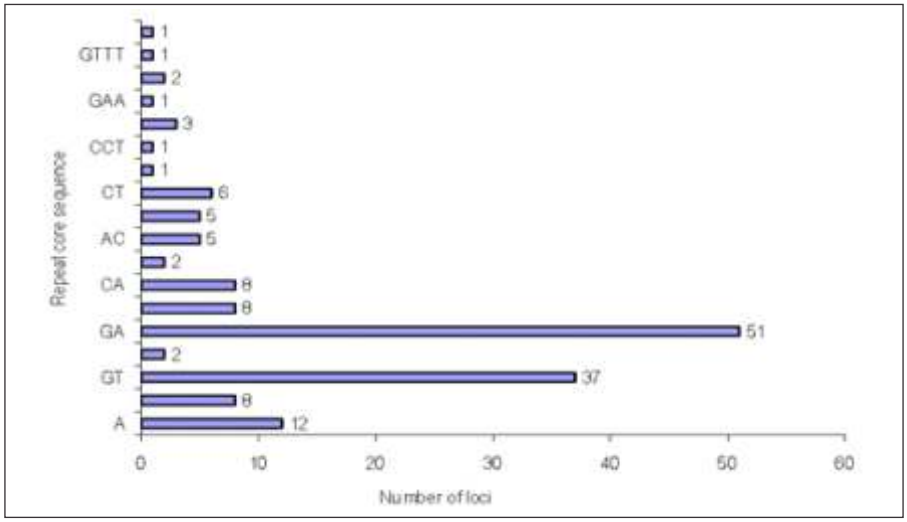


Figure 2.Frequency of the distribution of microsatellite repeat motifs in *Picrorhiza kurrooa* with different core sequences.

3.5 Knowledge Network on Medicinal Plants

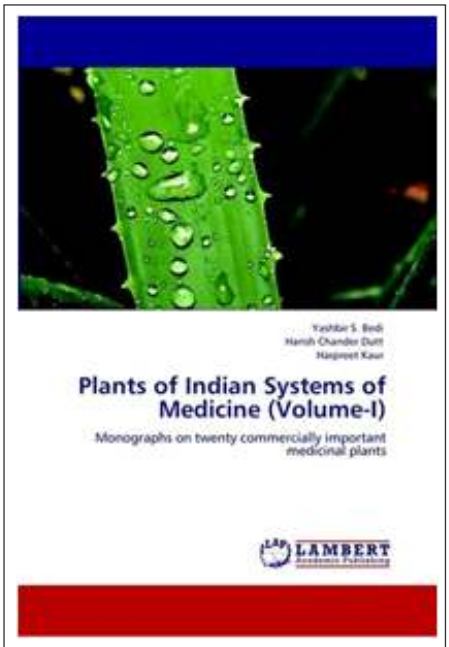
Harish Chander Dutt, Harpreet Kaur and Yashbir S. Bedi

Under ICMR sponsored project "Knowledge Network on Medicinal Plants" monographs on 40 Indian medicinal plants prioritized by National Medicinal Plants Board, New Delhi, were prepared. The 40 plants covered under the project were *Aconitum ferox* Wall.ex Ser., *Aconitum heterophyllum* Wall. ex Royle., *Acorus calamus* L., *Aegle marmelos* Corr., *Aloe barbadensis* Mill., *Andrographis paniculata* (Burm.f.) Wall.ex Nees., *Asparagus racemosus* Willd., *Bacopa monnieri* (L.) Pennell., *Berberis aristata* DC., *Bergenia cilliata* (Haw.) Sternb., *Boerhaavia diffusa* L., *Boswellia serrata* Roxb., *Cassia angustifolia* Vahl., *Centella asiatica* (L.) Urb., *Chlorophytum borivilianum* Sant. & F., *Coleus barbatus*Benth., *Commiphora wightii* (Arn.) Bhandari, *Crocus sativus* L., *Embelia ribes* Burm. f., *Emblica officinalis* Gaertn., *Garcinia indica* Choisy, *Gloriosa superba* L., *Glycyrrhiza glabra* L., *Gymnema sylvestre* R.Br., *Inula racemosa* Hook.f, *Nardostachys jatamansi* DC., *Ocimum sanctum* L., *Phyllanthus amarus* Schumach. &Thonn., *Picrorhiza kurrooa* Royle ex Benth., *Piper longum* L., *Plantago ovata* Forssk., *Rauvolfia serpentine* Benth. ex Kurz., *Santalum album* L., *Saraca asoca* (Roxb.) De Willd., *Saussurea lappa* C.B. Clarke, *Solanum nigrum* L., *Swertia chirata* Buch.-Ham.ex Wall., *Tinospora cordifolia* (Willd.) Hook. f. & Thomson, *Vitex negundo* L. and *Withania somnifera* (L.)Dunal

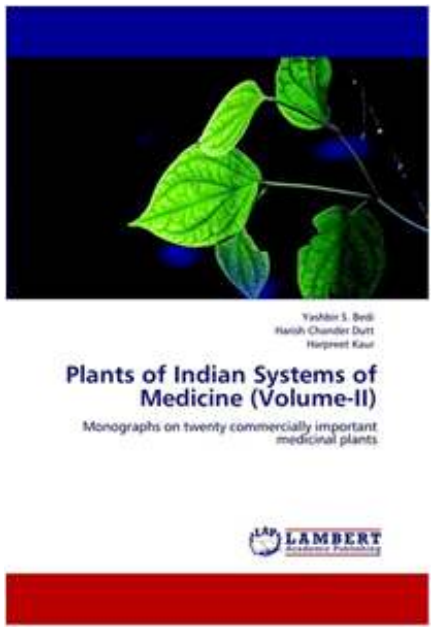
The monographs were prepared from data mined from books, pharmacopeias, research publications, CAB abstract, biological abstracts, chemical abstracts, medicinal and aromatic plant abstracts, journals. Further, online databases like SciFinder, IPNI, ARS-GRIN, TROPICOS, NISCAIR and websites of reputed organization like FRLHT, ICS-UNIDO etc. were also used extensively.

Each monograph encompasses details about the botanicalaspects (botanical name, synonyms, family, common name, habitat, morphology, part used as crude drug, morphological characteristics of the drug, substitutes and adulterants, cultivars, cultivation, ecotypes and cytotypes), traditional knowledge (ethnobotanical / folk lore / household remedies), chemistry (chemical markers, molecular markers and isozymes), pharmacology (bioactivity, clinical studies and toxicity), formulations (general usage and dosage), commercial aspects (production, demand, market trends, trade resources and major users), and patents and complete bibliography. Based on the analysis of data included under these monographs, it has been seen that there is a dire need to fill up the gaps with respect to data pertaining to chemical as well as molecular markers and commercial aspects for majority of these plants.

The final report submitted to ICMR has been published in two volumes as under:



Plants of Indian Systems of Medicine (Volume-I): Monographs on twenty commercially important medicinal plants (2011). LAP LAMBERT Academic Publishing.(*pp* 492).



Plants of Indian Systems of Medicine (Volume-II): Monographs on twenty commercially important medicinal plants (2011). LAP LAMBERT Academic Publishing.(*pp* 420).

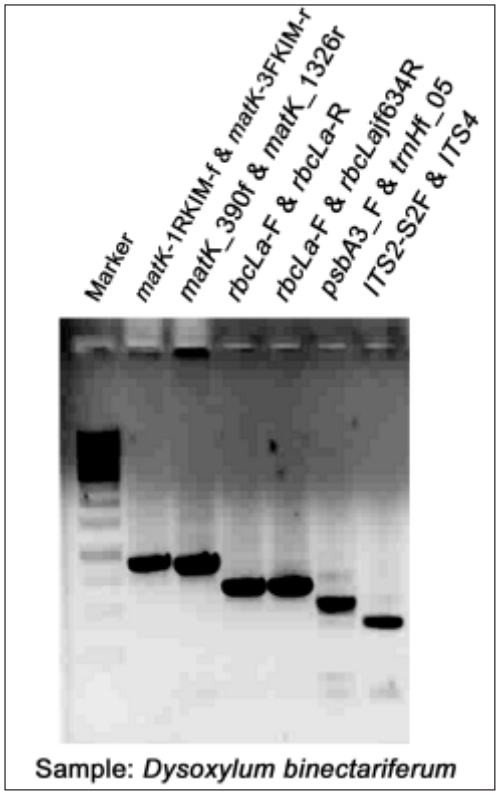


Figure 3. Amplification of DNA barcoding loci from *Dysoxylum binectariferum* Hook.f

3.6 Identification and Molecular Cloning of Cytochrome P450 genes from Coleus forskohlii Anet.

Praveen Awasthi, Yashbir S. Bedi, Ram Vishwakarma and Sumit G. Gandhi

Coleus forskohlii Briq.is a perennial herb from mint family, which grows in subtropical temperate climates. Traditional medicinal uses of *C. forskohlii* include treatment of digestive disorders, skin ailments, genito-urinary disorders, respiratory problems, gastric pain, circulatory and heart ailments, hypertension etc. Chemoprofiling of *C. forskohlii* have found accumulation of several labdane diterpenes in its roots of C. forskohlii, which are responsible for these medicinal properties. Coleonol (forskolin), the principle bioactive component, is a reversible and a very strong activator of adenylate cyclase. Forskolin has a very unique chemical structure, with eight chiral centers and diverse functional groups. Only two genes involved in biosynthesis of common upstream intermediates of the terpenoid pathway have been cloned from *C. forskohlii.* Chemical structures of advanced intermediates of forskolin biosynthesis suggest that about 4-5 Cytochrome P450 monooxygenases could be involved in catalyzing the final reactions. Cytochrome P450 monooxygenases are Heme proteins, involved in various biosynthetic and detoxicative pathways in plants. We have designed a series of degenerate primers from conserved regions of CYP450 sequences and have created libraries from root and leaf cDNA of *C. forskohlii.* A total of 800 positive clones were picked and screened for redundancy (Figure 1). After sequencing, we obtained 44 CYP450 sequences. CAP3 assembly of these sequences resulted in 31 singletons and 4 contigs. Alignment with other CYP450 members helped to identify the likely subfamily to which these sequences belong. (Table 1).

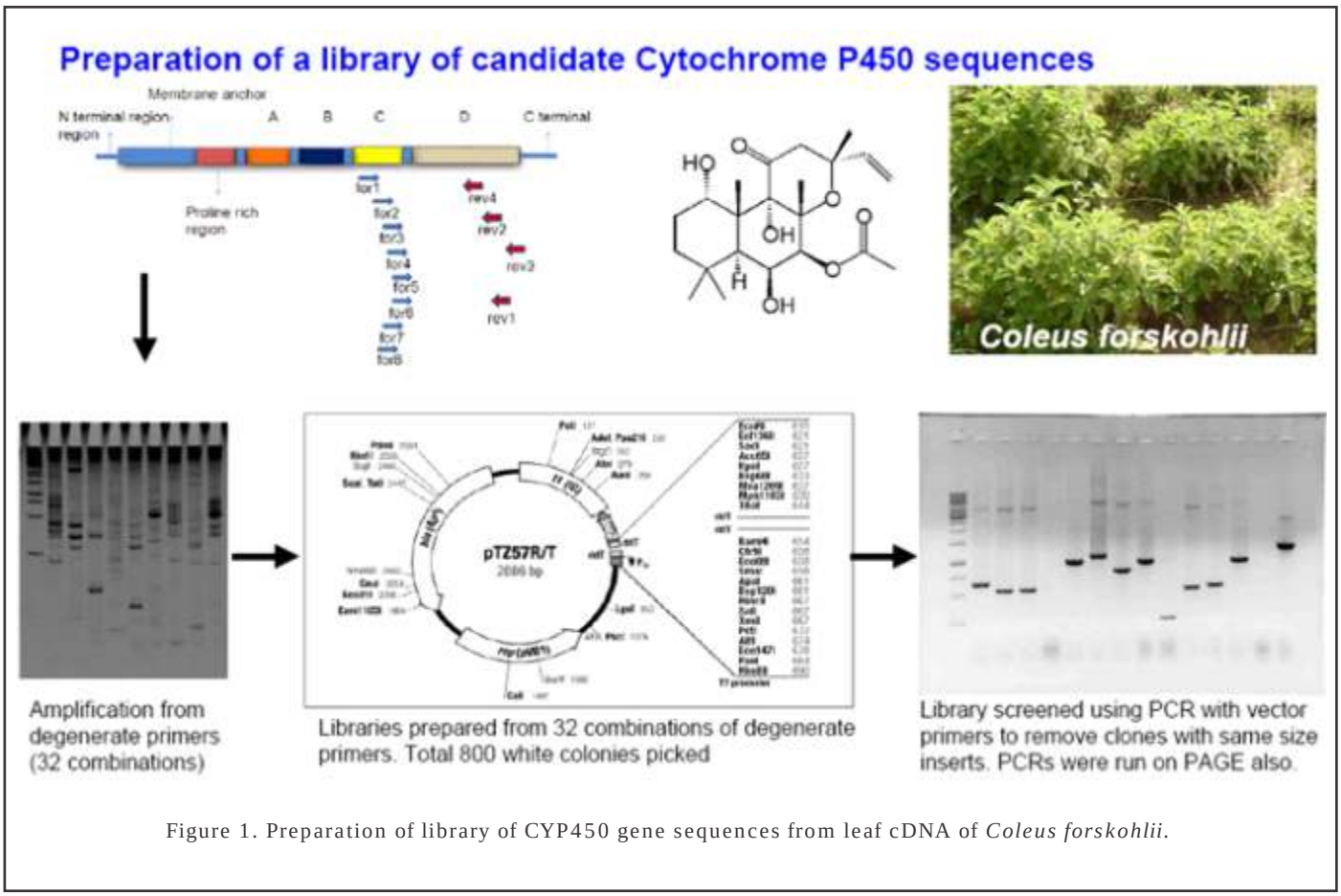


Figure 1. Preparation of library of CYP450 gene sequences from leaf cDNA of *Coleus forskohlii.*

S.NO	CLONE CODE	BLASTX MATCH
1	PRL134	CYP73A
2	PRL4	CYTOCHROME P450
3	PRL11	CYP83A
4	PRL12	CYP706/CYP93A
5	PRL18	CYP734/CYP72A65
6	PRL25	CYP706/CYP93A
7	PRL27	CYP98A2
8	PRL39	CYP81D
9	PRL48	CYP81D
10	PRL76	CYP734A1
11	PRL80	CYP706/CYP93A
12	PRL89	CYP734A1
13	PRL90	CYP98A2
14	PRL19	CYP81D
15	PRL23	CYP706
16	PRL28	CYP81D
17	PRL88	CYP81D
18	PRL147	CYP81A
19	PRL157	CYP706
21	PRL165	CYP98A2
22	PRL424	CYP93A

S.NO	CLONE CODE	BLASTX MATCH
23	PRL425	CYP93A1
24	PRL439	FLAVONE SYNTHASE-II
25	PRL547	FLAVONOID 3' MONOOXYGENASE
26	PRL548	p-coumaryl shikimate 3' hydroxylase
27	PRL559	cyp76
28	PRL452	p-coumaryl shikimate 3' hydroxylase
29	PRL483	FLAVONOID 3' MONOOXYGENASE
30	PRL497	CYP706
31	PRL570	p-coumaryl shikimate 3' hydroxylase
32	PRL576	FLAVONOID 3' MONOOXYGENASE
33	PRL265	p-coumaryl shikimate 3' hydroxylase
34	PRL258	p-coumaryl shikimate 3' hydroxylase
35	PRL205	p-coumaryl shikimate 3' hydroxylase
36	PRL216	p-coumaryl shikimate 3' hydroxylase
37	PRL206	p-coumaryl shikimate 3' hydroxylase
38	PRL214	p-coumaryl shikimate 3' hydroxylase
40	A1.1C	Flavonoid synthase
41	A1.2Q	Flavonoid synthase
42	A3.2e	Flavonoid 3' Monoxygenase
43	C3.1d	CYP71
44	C3.2c	CYP76

Table 1 CYP450 clones obtained from leaf cDNA of *C. forskohlii* and likely genes / CYP families to which these sequences belong.

3.7 Cloning of gene encoding antifungal protein OSMOTIN from *Ocimum basilicum* Linn.

Irshad Ahmad, Yashbir S. Bedi and Sumit G. Gandhi

Plants mount several defense responses against invasion by various pathogens. One such response includes synthesis of host-encoded, pathogen-related (PR) proteins. The PR proteins have been further classified on the basis of amino-acid sequence homology, serological relationships and biological activities. The PR5 subclass includes OSMOTINs, which accumulate at high levels in plant cells after NaCl adaptation (exposure to high salinity – osmotic stress). *Osmotin* genes are induced by several hormonal and environmental signals like NaCl, Abscisic acid (ABA), ethylene, wounding, viral infection, dessication, UV light and fungal infections. Both, *in-vitro* and *in-vivo* assays have demonstrated marked anti-fungal activities of OSMOTIN. Heterologously expressed OSMOTIN inhibits the growth of several fungi in agar diffusion assays while transgenic plants over expressing *Osmotin* show enhanced resistance to infections by phytopathogenic fungi.

We have identified *Osmotin* related gene sequences from the EST resource of *Ocimum basilicum*. Primers were designed from the EST fragments and amplification of partial *Osmotin* gene was obtained from the genomic DNA. The amplicons were eluted from gel and sequenced. Specific primers were designed for the sequences. RNA was isolated from the leaves of the plant. 3' and 5' RACE was performed for full length cloning of the *Osmotin* gene (Figure 1).

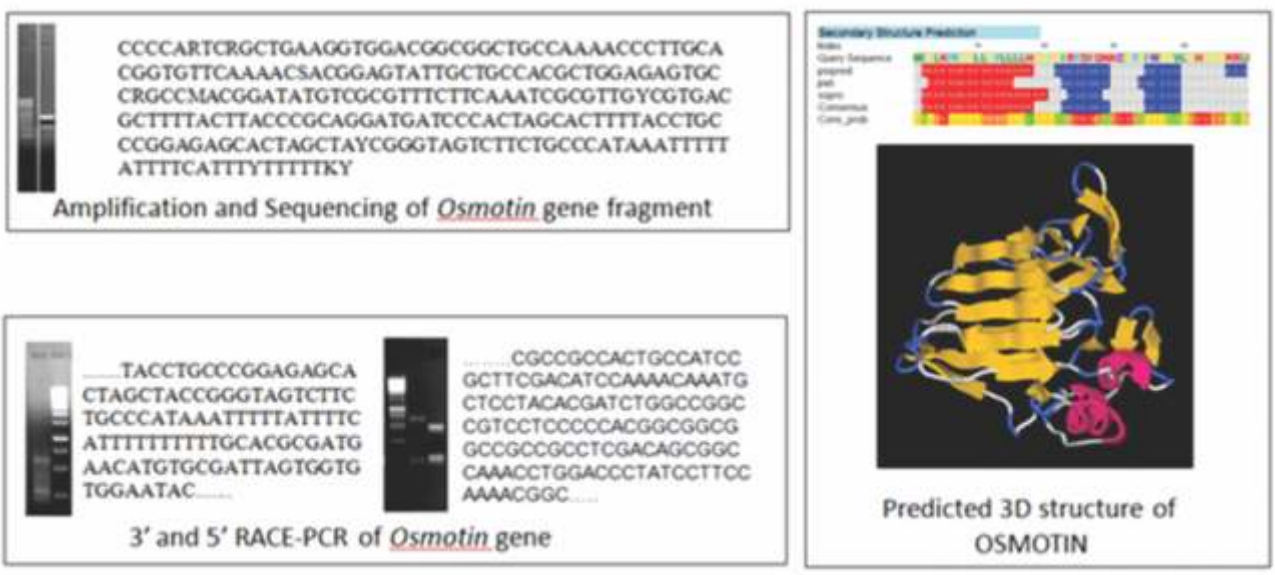


Figure 1. Cloning and sequencing of Osmotin gene from *Ocimum basilicum* Linn. Amplification and sequencing of core fragment, 3' and 5' RACE amplicons of Osmotin gene from Ocimum basilicum. The full length CDS was theoretically translated and its 3D structure was modeled for prediction of regions which could have antifungal activity.

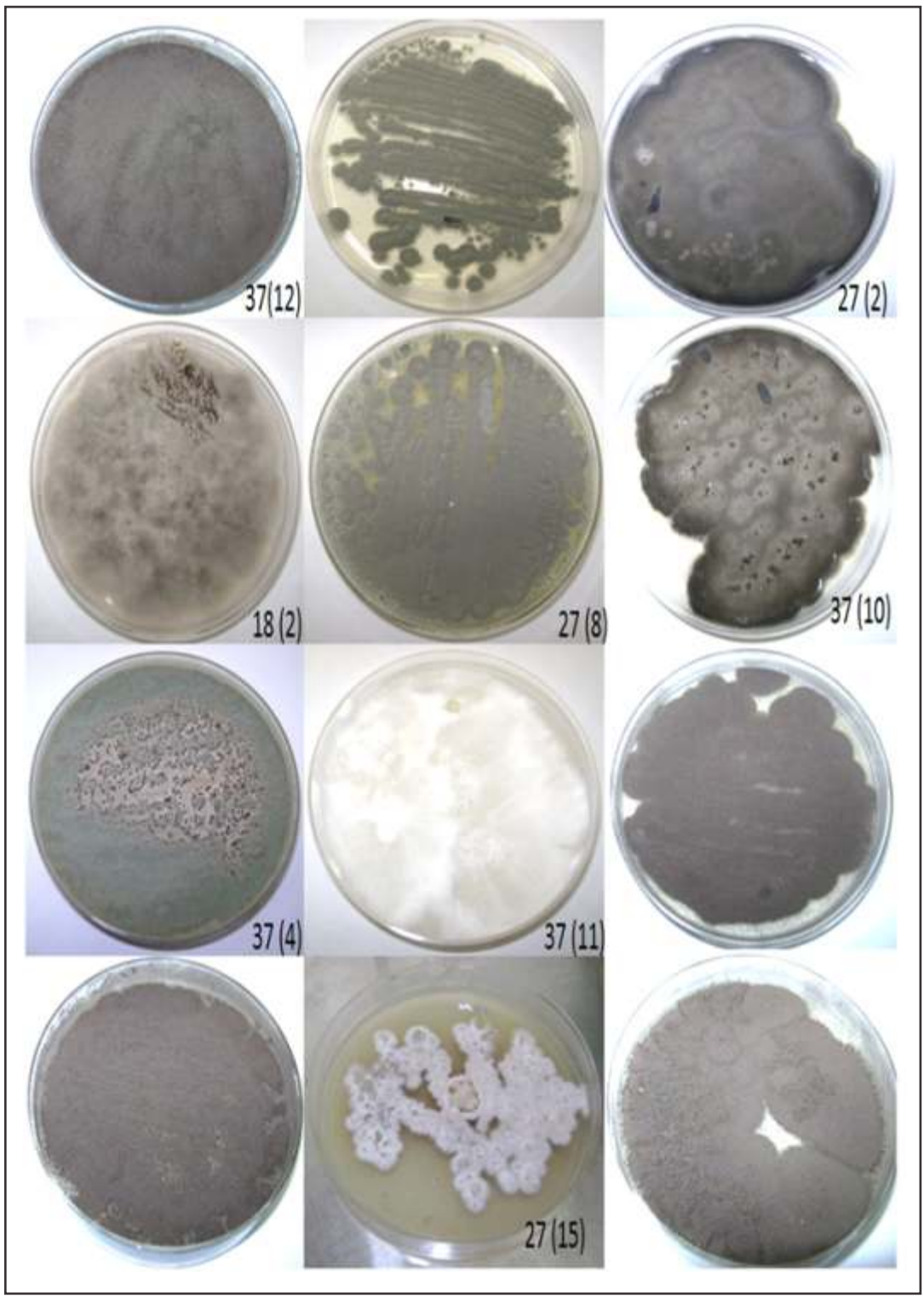
3.8 Understanding the affect of endophytes on artemisinin biosynthesis in *Artemisia annua* Linn.

Abid Mir Hussain, Vidushi Mahajan, Irshad Ahmad, Sumit G. Gandhi and Yashbir S. Bedi

Artemisia annua L. is a source of a potent anti-malarial drug: Artemissinin. Artemissinin and its derivatives are effectively used to treat cerebral malaria in human subjects with little side effects. Artemisinin is a secondary metabolite identified as a sesquiterpene lactone endoperoxide.

Symbiotic associations between living organisms are known to have played an important role in the evolution of present forms of life on earth. Plants include a community of endosymbiotic fungi that form intimate association with cells, tissues, cell cultures and regenerated plants. These fungi regulate plant growth and development and contribute genes and natural products that enable plants to adapt to changing environments. Several gene expression studies have demonstrated the induction or suppression of various transcription factors on interaction with microbes or their extracts.

We have isolated and characterized endophytes associated with leaves of *Artemisia* annua. A total of 16 representative isolates of endophytic fungi and bacteria were obtained (Figure 1). 16s rDNA and ITS sequencing were used for molecular identification of bacterial and fungal isolates respectively (Figure 2). Further, morphological characterization of fungal isolates was also carried out. Autoclaved and filtered extracts of fungal isolates were prepared and added at three different concentrations into tissue cultures of *Artemisia*. Transcription analysis of key artemisinin biosynthesis and regulatory genes was carried out using RT-PCR (Figure 3).



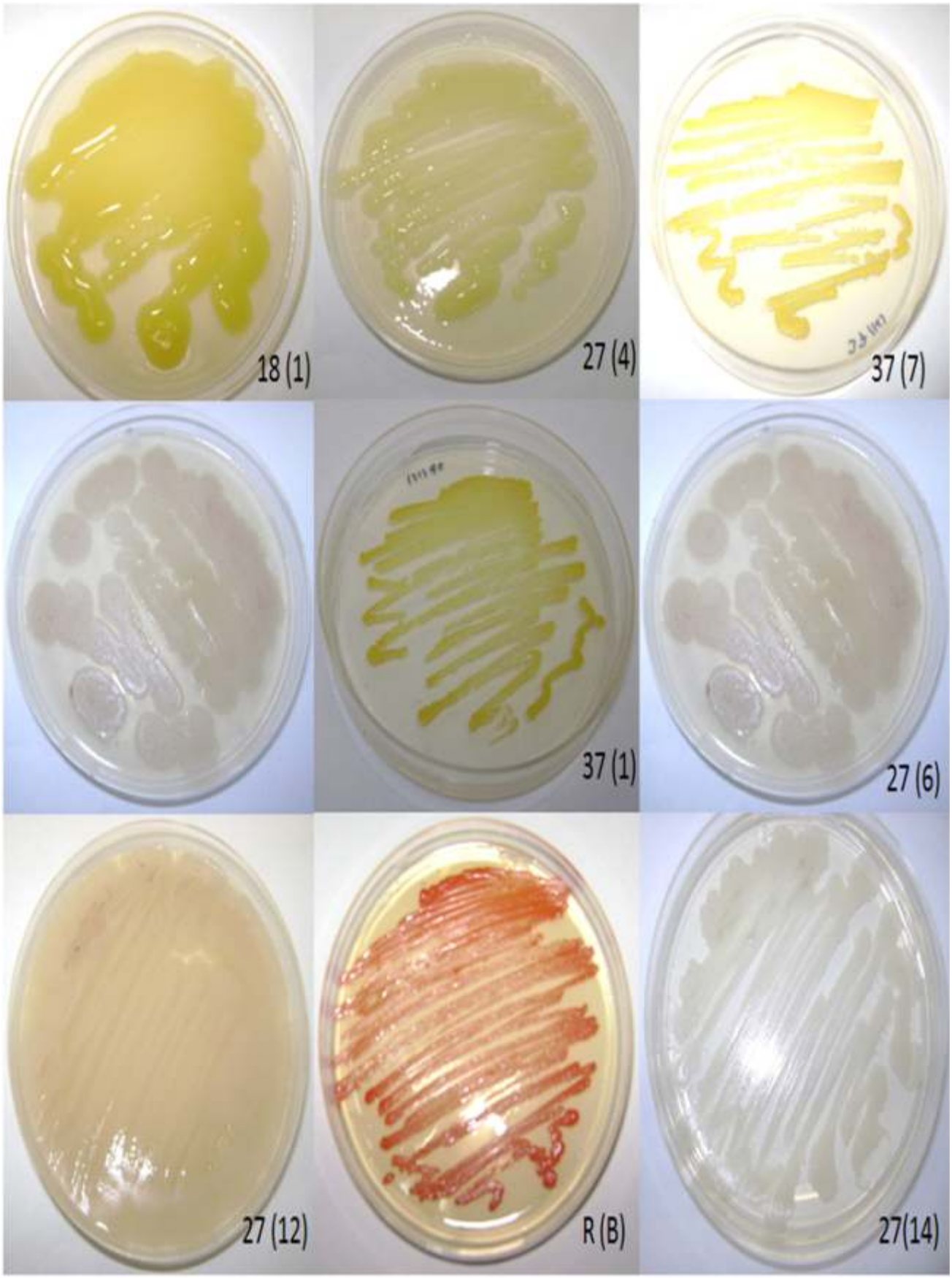
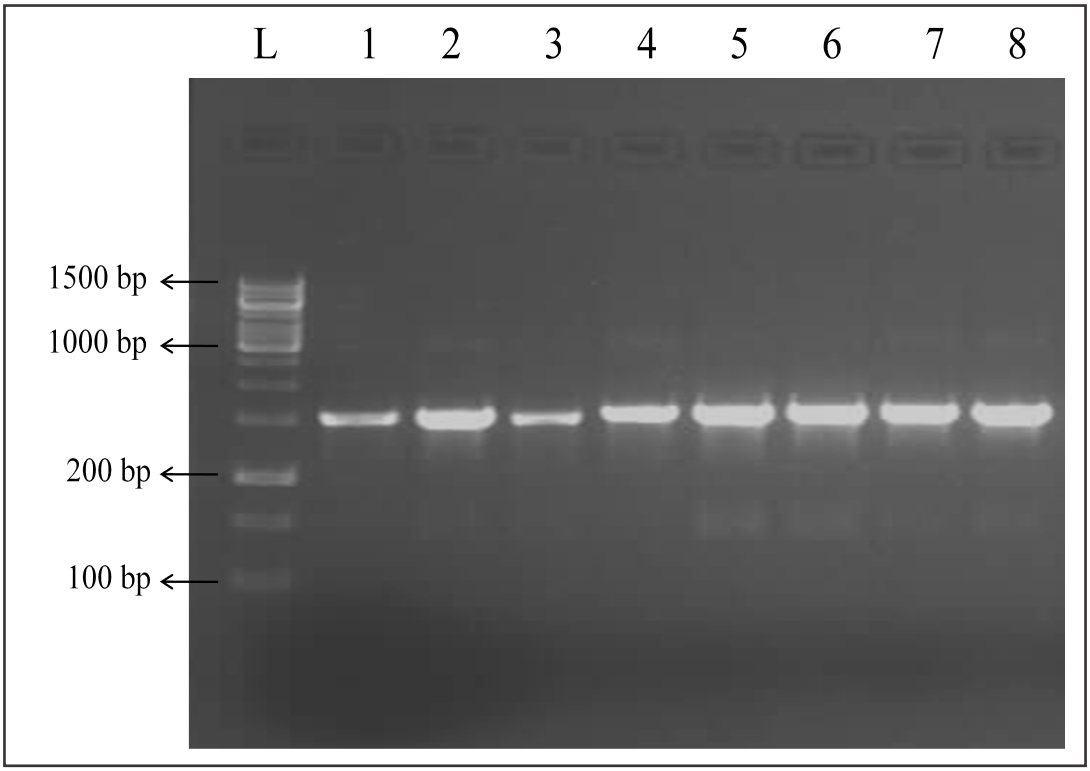


Figure 1. Endophytic fungi and bacteria isolated from leaves of *Artemisia annua* L.



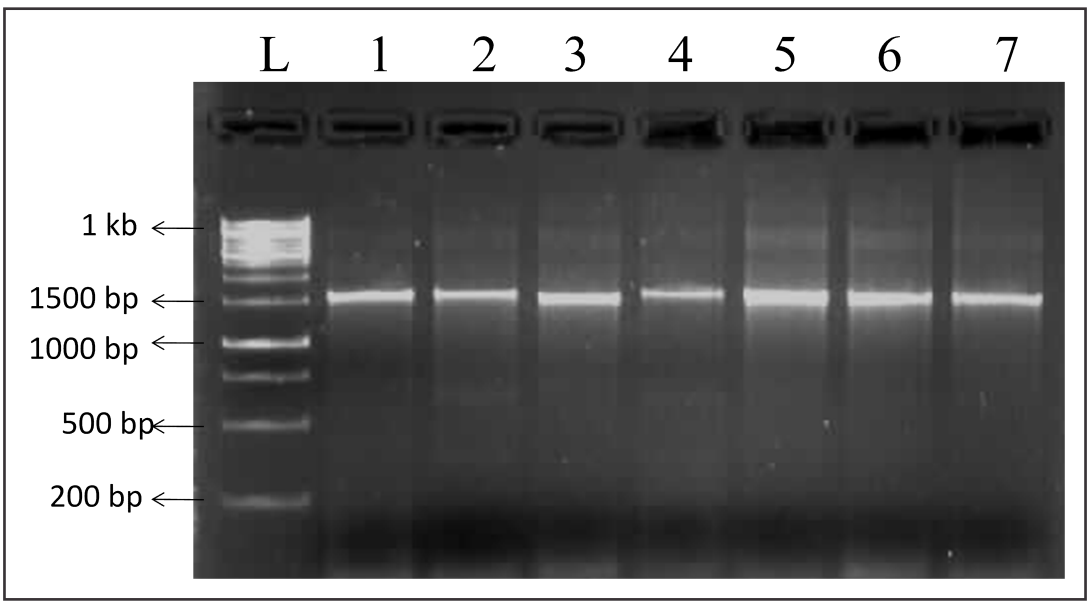


Figure 2. PCR amplification of Internal Transcribed Spacers and 16s rDNA from endophytic fungi and bacteria respectively.

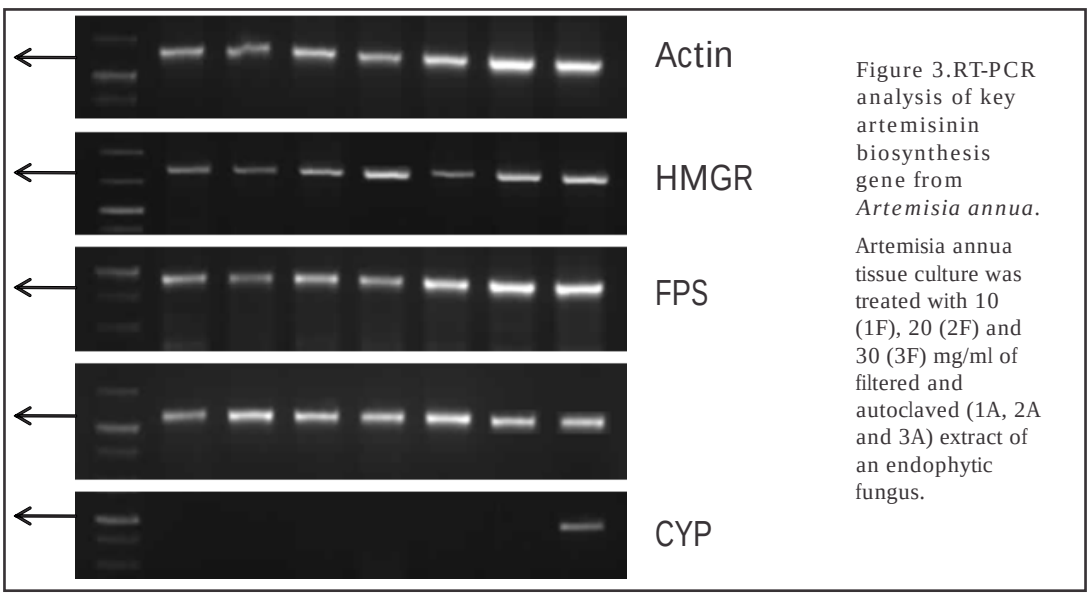


Figure 3.RT-PCR analysis of key artemisinin biosynthesis gene from *Artemisia annua*.

Artemisia annua tissue culture was treated with 10 (1F), 20 (2F) and 30 (3F) mg/ml of filtered and autoclaved (1A, 2A and 3A) extract of an endophytic fungus.

3.9 Co-assesment of variation in genic microsatellites and essential oil composition in *Ocimum* species

Irshad Ahmad, Praveen Awasthi, Vidushi Mahajan, S.R. Meena, Yashbir S. Bedi and Sumit G. Gandhi.

Ocimum spp. are important economic and medicinal herbs with proven efficacy in Unani and Ayurvedic systems of medicine. Essential oils from *Ocimum* are highly valued in perfumery, flavouring and pharmaceutical industries. Pharmacological activities of *Ocimum* oils include calcium-channel blockade, ameliorative effects in axotomy-induced neuropathy, controlling the alteration of neurotransmitter levels due to noise stress, antinociceptive, analgesic and spasmolytic activities. In spite of such an array of pharmacological activities and importance in traditional medicine systems irregularities have been noticed by several researchers in taxonomy and total number of species in *Ocimum* genus. In the present study we have developed and used EST-SSR markers to determine the genetic relatedness between 7 different species of *Ocimum*. About 23,000 EST sequences from *Ocimum basilicum* were downloaded from NCBI database and assembled using CAP3. Simple sequence repeats (SSRs) were screened in these sequences using standalone perl scripts. Primers were designed in the conserved flanking regions. 25 EST-SSR primer pairs were randomly selected and used for amplification across seven species of *Ocimum*. Further essential oils were extracted from these seven species and their compositions were determined using gas chromatography. We have assessed amplicon size variation of EST-SSR markers along with variation in essential oil composition.

Parameter	Value
Total number of ESTs searched	23,260
Total number of ESTs with SSRs	471
Total number of ESTs with single SSR	451
Total number of ESTs with >2 SSRs	20
Repeat Type	
Mononucleotide	166 (33.74%)
Dinucleotide	122 (24.80%)
Trinucleotide	131 (26.63%)
Tetranucleotide	15 (3.05%)
Pentanucleotide	17 (3.46%)
Hexanucleotide	31 (6.30%)
Heptanucleotide	10 (2.03%)
Total number of SSRs identified	492
Total length of Unigenes searched (kb)	~ 9,754 kb
Frequency of SSRs	1 per 19.82kb

Figure 1.Summary of EST-SSR computational search results.

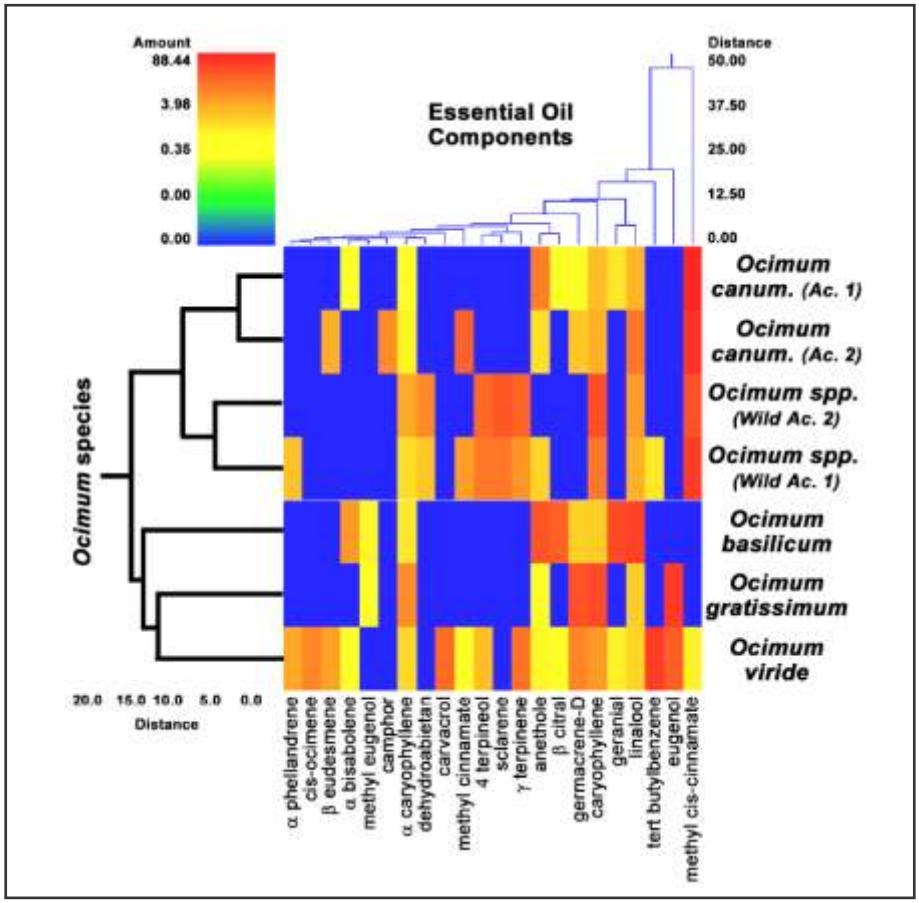


Figure 2. Clustering of Ocimum species / accessions based on essential oil composition

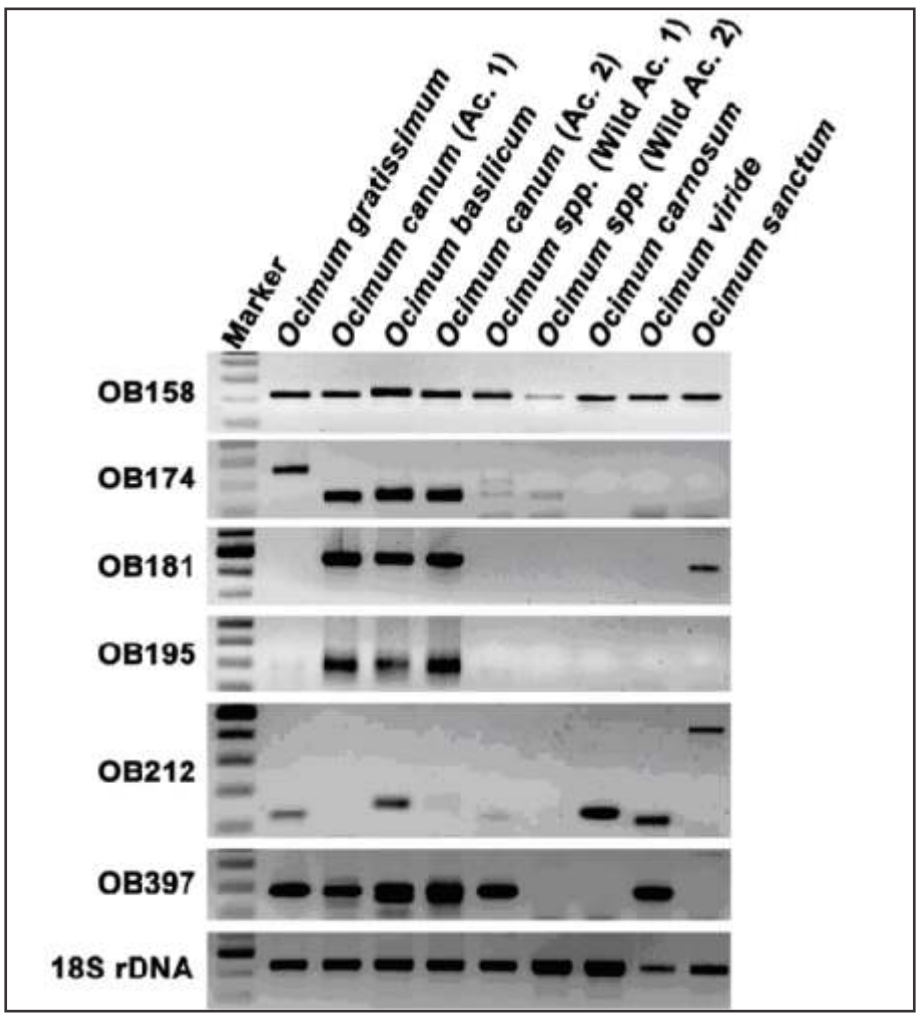


Figure 3. PCRs using new EST-SSR markers developed for use in *Ocimum* species.

3.10 Molecular cloning, bacterial expression and promoter analysis of squalene synthase from Withania *somnifera* (L.) Dunal- an Indian ginseng

Wajid Waheed, Sumeer Razdan, Rekha S. Dhar, Surrinder K. Lattoo and Ram Vishwakarma

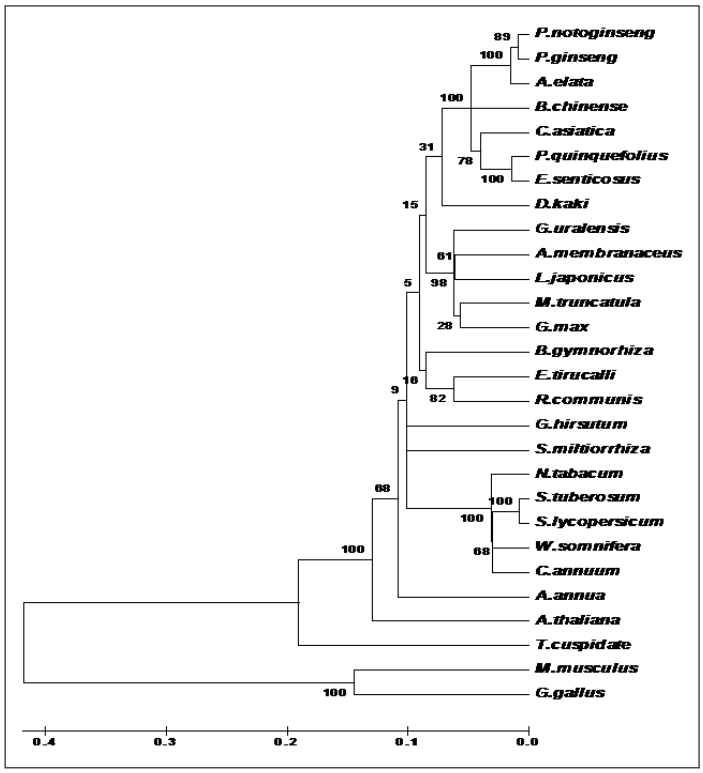
A full length cDNA of *SQS* gene was obtained from the leaf tissues of withaferin-A rich chemovariant of *W. somnifera* by degenerate PCR and RACE methods (NCBI GenBank Acc. No. GU474427; Protein ID. ADC95435.1). A 450 bp core fragment was obtained from the initial RT/PCR reaction. To obtain 5' and 3' ends of core amplified fragment RACE technique was used. Gene specific primers used for carrying out RACE were designed using partial length sequence obtained from amplified core fragment. After assembling 5' and 3' RACE sequences, a cDNA sequence of 1581 bp with a poly-A tail was obtained which contained an open reading frame of 1236 bp encoding a protein of 411 amino acids (Fig. 1). A 173 bp 5´ un-translated region (UTR) was upstream of the start codon and the coding region was followed by a 152 bp 3´UTR (Fig. 1).

The C-terminal hydrophobic region was also predicted by TMHMM and SPLIT 4.0 bioinformatics programs. Secondary structure analysis of WsSQS protein by SOPMA program revealed that WsSQS consists of α-helixes (65.69%), β-turns (4.38%) joined by extended strands (8.03%), and 37 random coils (21.90%). Using human squalene synthase (1EzfB) as a template for comparative modelling, a predicted 3D structure was determined for WsSQS by applying I-TASSER simulation (Fig. 3a). The template shared 46.8% identities with the WsSQS sequence. Analysis of the evolutionary conservation of its surface amino acids was performed using ConSurf program. Several residues with high scores were found to be functional and structural residues in the protein by ConSeq servers (Fig. 3d).

Analysis of the deduced protein sequence

The calculated molecular mass and the predicted isoelectric point of the *WsSQS* deduced polypeptide sequence were 47.06 kDA and 7.91 respectively. The amino acid sequence of the Ws*SQS* shared 92% identity with that of *Capsicum annum*, *Solanum tuberosum* and *Solanum lycopersicum*, 91% with *Nicotianatabacum* and 90 % with *Nicotiana benthamiana.*

Fig.2 Neighbor joining phylogenetic tree constructed form the deduced amino acid sequences of *WsSQS* from *W. somnifera* and SQSs from other organisms using MEGA 5.05. Numbers above the branches indicate bootstrap values. WsSQS is red boxed. Members of the Solanaceae family are present in a separate clade shown in a bracket.



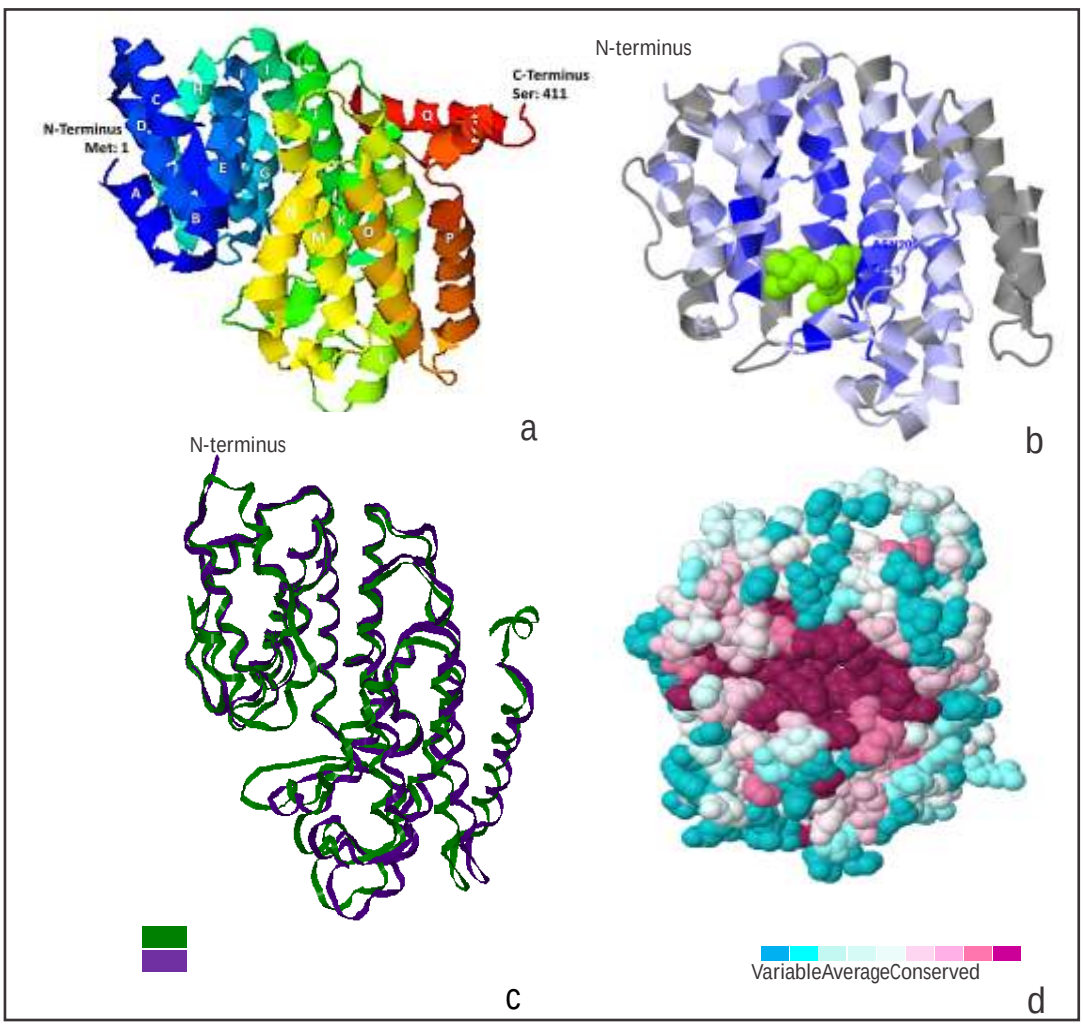


Fig. 3 Three dimensional models and conserved residue prediction for WsSQS. a; Cartoon display of the 3D structure of WsSQS as predicted by I-TASSER Web Server using crystal structure of human squalene synthase(PDB accession code 1EZFB) as template. The main α helices are numbered with A-Q from N-terminal (Methionine 1) to C-terminal (Serine 411). b; Predicted ligand (shown in green) binding site as predicted by 3DLigandSite Web Server. Active site is predicted to involve amino acids GLU 80, ASP 81, ASN 209, ILE 210. Superimposition of 3D ribbon model of WsSQS (Green) with human SQS (Purple) using 3d-SS superimposition service.Conserved residue analysis of WsSQS using Consurf and Conseq Web Servers. Residue conservation from variable to conserved is shown in blue(1) to violet(9). The residues involved in substrate binding and active site are shown in the center core of the structure.

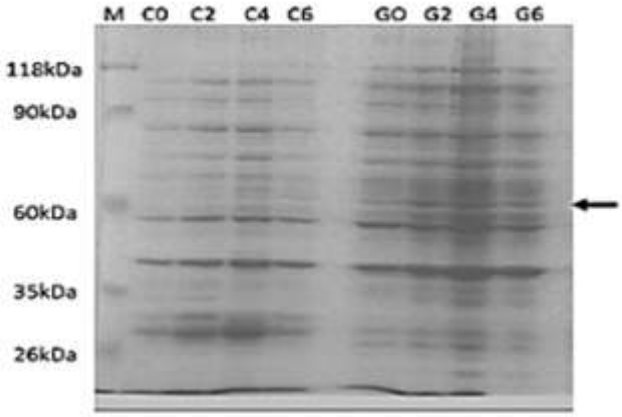


Fig. 6 Time course expression of truncated *WsSQS* gene (pGEX-*WsSQSTrunc*) in *E. coli* BL21 (DE3). pGEX-*WsSQSTrunc* is a 24 amino acid, C-terminus deleted *WsSQS* gene fused *in*-frame with GST tag. Total protein extracts of E. coli cells were used for detection by SDS-PAGE and stained with Coomassie blue.Lane M, Protein Molecular Weight Markers; Lane C0-C6 , induction of control (vector only) 0 (C0),2 (C2),4(C4) and 6(C6) hours after induction. Lane G0-G6, induction of recombinant protein after 0(G0), 2 (G2), 4(G4) and 6(G6) hours induction with 1mM IPTG at 37°C. The arrow indicates the position and size of the recombinant *WsSQS* protein(65kDa).

Expression of *WsSQS* in *E. coli*

In order to express *WsSQS* in *E. coli*, we cloned the entire coding region of the *WsSQS*cDNA, as well as a truncated *WsSQS* cDNA having a deletion of the last 25 amino acids at the carboxyl-terminus into pGEX4T2 vector, an expression vector with *Tac* promoter and a GST-tag yielding pGEX4T2-*WsSQS and* pGEX4T2-*WsSQSTrunc* respectively. The expression constructs were checked for in-frame fusion by DNA sequencing. The geneconstructs were transformed into competent BL21 cells and their expressions were induced by the addition of 1 mM IPTG at approximately OD600 = ~0.5. Low levels of the *SQS* polypeptide were observed in *E. coli* that contained the putative full-length *SQS* cDNA (pGEX4T2-*WsSQS*) (data not shown). In contrast, readily measurable levels of *SQS* polypeptide were observed in the extracts of *E. coli* that expressed the truncated *WsSQS* (pGEX4T2-*WsSQSTrunc*). This resulted in the appearance of a new fusion polypeptide with an expected molecular mass of approximately 65 kDa (40-42 kDa *WsSQS* plus 25 kDA GST-tag) when resolved on SDS-PAGE.

High levels of expression of the truncated *WsSQS* protein were observed 1 h after addition of 1 mM IPTG (Fig. 6).

Results indicated that *WsSQS* expressed in a constitutive manner in the tissues of roots, stalks and leaves, with the highest expression in leaves (Fig. 8). The expression pattern of

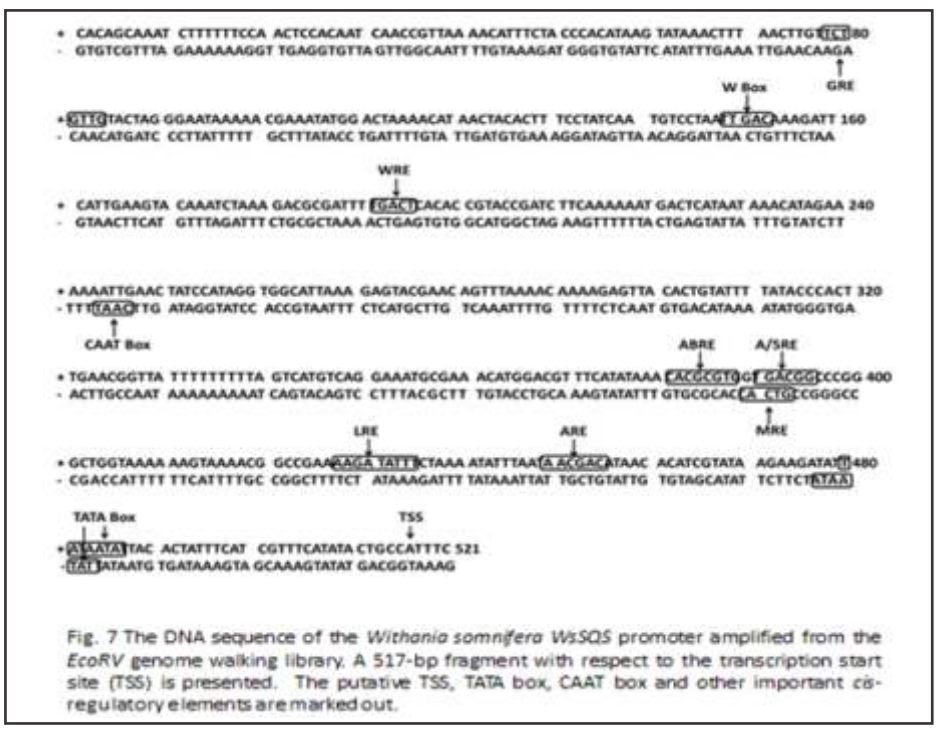


Fig. 7 The DNA sequence of the *Withania somnifera WsSQS* promoter amplified from the *EcoRV* genome walking library. A 517-bp fragment with respect to the transcription start site (TSS) is presented. The putative TSS, TATA box, CAAT box and other important *cis*-regulatory elements are marked out.

Expression profile of *WsSQS* in different tissues

To investigate the *WsSQS* expression pattern in different tissues of *W. somnifera*, total RNAs extracted from roots, stalks, and leaves were used as the template in quantitative RT-PCR analysis.

WsSQS in different tissues corroborated well with the squalene content in the respective tissues (data not shown).

Isolation and Analysis of *WsSQS* promoter region

Using genome walking, we isolated a 693 bp fragment upstream of the start

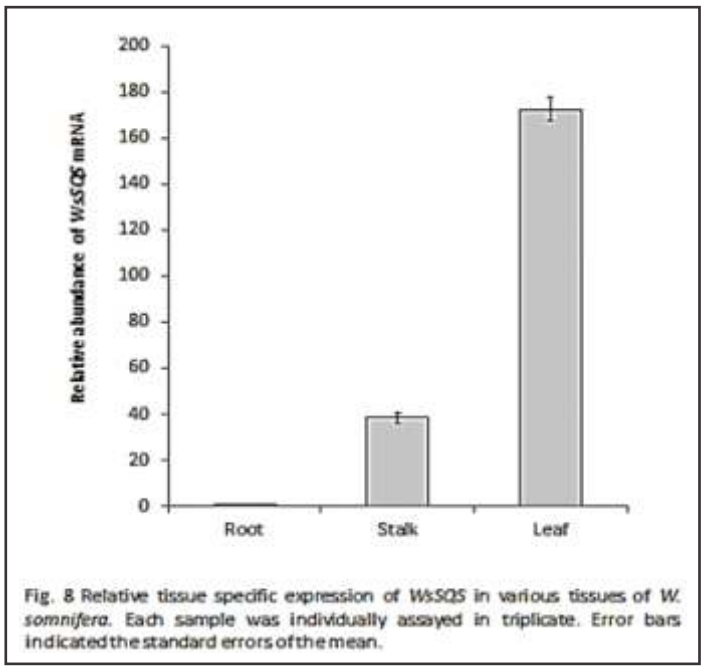


Fig. 8 Relative tissue specific expression of *WsSQS* in various tissues of *W. somnifera*. Each sample was individually assayed in triplicate. Error bars indicated the standard errors of the mean.

codon including the 173 bp 5´ UTR which corresponded to the putative promoter region of *WsSQS* (Fig. 7). It possessed a typically high A+T content (65.46%) commonly found in other plant promoters. Computational analysis using PlantCare and PLACE databases revealed several *cis*-elements including eight potential TATA box sequences within the promoter region of *WsSQS* at positions 445, 466, 476, 477, 478, 479, 481 and 484. Another consensus eukaryotic *cis* -element CAAT box, was also observed at positions 5, 147, 138, 243, 136, 170, 146, 161, 26, 242 and 137. Furthermore, several stress related *cis*-elements were also identified in the promoter region of *WsSQS*. Four Myb (at positions 33, 78, 324 and 30) and two Myc (at positions 136 and 316) recognition sites were also identified. W-boxes present in the promoters of many defence related genes of other plants were also found in the promoter region of *WsSQS* at positions 150, 341, 346, 191, 220 and 390. The WRKY transcription factors which are involved in various physiological processes including pathogen defense bind specifically to these W-boxes.

Low temperature responsiveness and heat stress responsive element were also identified. Light responsive *cis*-elements, 3-AF binding site (position 424), GT1CONSENSUS (positions 237, 349, 403, 12 and 13) and GATA box (positions 428, 474,133 and 250) were also present in the promoter region. One core motif, GARE described as dehydration and GA responsive expression element (Sutoh and Yamauchi 2003) was also identified at position 77.

The transcription start site (TSS) of the *WsSQS* promoter was determined by 5´ RACE strategy. More than 10 RACE products were sequenced and the TSS of the *WsSQS* mRNA was identified at the site “A”, 31 bp downstream of the Predicted TATA box (Fig.8).

Effect of phytohormones on withanolides biosynthesis

To validate the predicted *cis*-acting elements, the effect of MeJA, SA and 2, 4-D treatments on the biosynthesis of a key withanolide, withaferin-A was analysed. *Withania* plants were mist sprayed with SA (1 mM), 2, 4-D (0.05 mM) and MeJa (0.1 mM) solution on both sides of the leaf until liquid dripped from the leaves. Leaf tissues were collected after 12 and 24 h after treatment and phytochemical analysis was done. Similar results were also observed when *W. somnifera* was treated with 0.1 mM SA, an important component of signal transduction cascades activating plant's defense response against pathogen attack (Durner et al. 1997), and increase in the biosynthesis of withaferinA was more prominent compared to MeJA. With 2, 4-D, phytohormone known for inducing cell division and other growth related physiological phenomenon in plant cells, a slight increase in withaferin-A levels was also observed (Fig. 9). This might be because of the fact that auxins have been shown to upregulate DWARF4 gene of brassinosteroids biosynthetic pathway increasing the brassinosteroids levels (Chung et al. 2011). Since brassinosteroids pathway shares a considerable homology with withanolides biosynthetic pathway, it is possible that DWARF4 gene product may be a common precursor to both brassinosteroids and withanolides. All these results tend to be in agreement with the *WsSQS* promoter analysis and indicate that *WsSQS* is signalling molecules-responsive gene, which may be involved in the defence responses and regulation of secondary metabolites in *W. somnifera.*

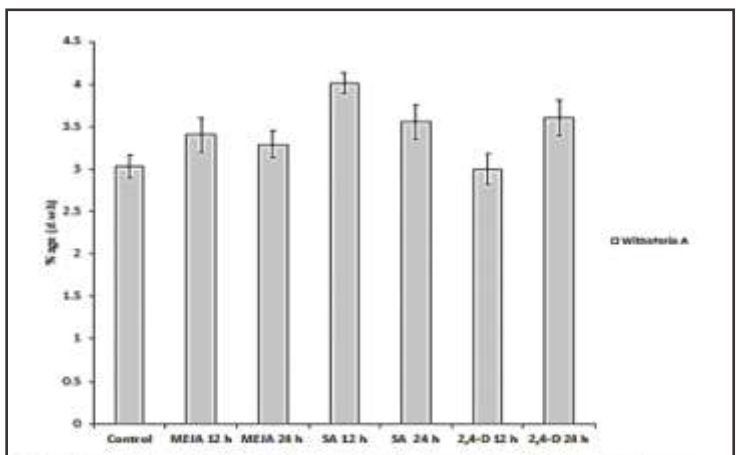


Fig. 9 Up-regulation of withaferin A content in *W. somnifera* by different phytohormones. Plants were sprayed with Methyl-jasmonate (0.1 mM MeJa), Salicylic acid (1mM SA) and 2, 4-Dichlorophenoxy acetic acid (0.05 mM2, 4-D) dissolved in DMSO. Control plants were sprayed with equal concentration of DMSO as that of treatments. Data were collected 12 and 24 hours after treatment.

3.11 Molecular characterization and identification of *cis*-regulatory elements of squalene monooxygenase gene from Ashwagandha *(Withania somnifera)*

Sumeer Razdan, Wajid Waheed, Surrinder K. Lattoo, Rekha S. Dhar and Ram Vishwakarma

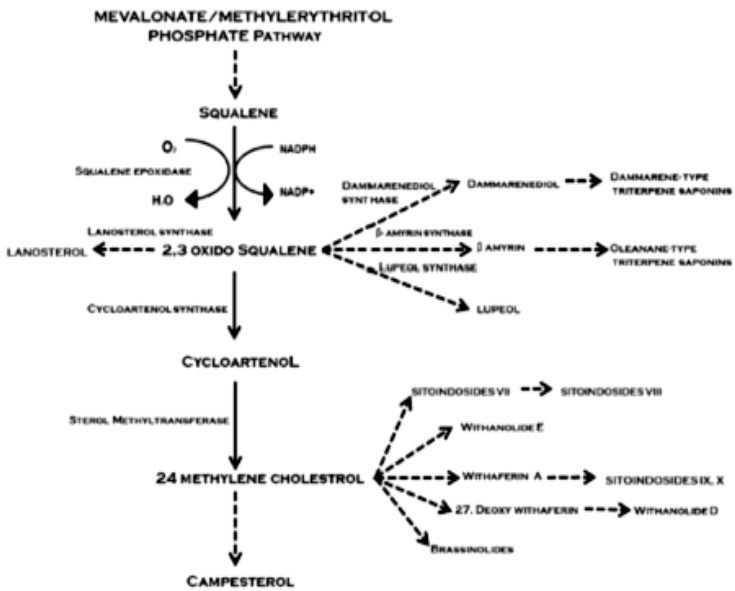


Fig. 1 Putative biosynthetic pathway of withanolides; dashed arrows represent multiple steps.

A Full length gene designated as *WsSQE (*NCBI GenBank accession no.GU574803was isolated from the Withaferin A rich accession of *W. somnifera.* Based upon the conserved amino acid domains of squalene epoxidase from other plant species, degenerate primers were designed to amplify an initial core fragment of 550 bp. RACE technique was used to obtain the 5' and 3' ends of core fragment. Partial length sequence of core amplicon was used for designing the gene specific primers

(GSP). Utilizing the GSP, RACE amplicons of 5' and 3' ends were amplified, sequenced and assembled, generating a full length gene sequence of 1900 bp containing an ORF of 1596 bp encoding a protein of 531aa (Fig.2) The 5' and 3' UTR sequences were of 165 bp and 195 bp res pectively (Fig. 2).

Analysis of the deduced protein sequence

Based upon sequence homology tool (Blastp), deduced amino acid sequence of *WsSQE* revealed homology with other plants such as *Datura innoxia* (95%) *Medicagotruncul ata* (87%), *Aralia elata* (81%) *Medicago sativa* (80%) *Panax notoginseng* (80%), *Gynostemma Pentaphyllum* (81%), *Euphorbia tirucalli* (78%), *Panax ginseng* (81%), *Eleutherococcus senticosus* (80%), *Arabidopsis thaliana* (75%)(Fig. 3). A phylogenetic tree was constructed using CLUSTALW 2 tool to ascertain the degree of evolutionary relatedness among different plant species (Fig. 4). The

degree of relatedness correlated well with the amino acid similarity among these proteins. Using the SMART tool, *WsSQE* protein sequence predicted several overlapping domains, which included DAO (D-amino acid oxidase) (66-375 amino acids), lycopene cyclase (66-428aa), squalene epoxidase (SE) (215-488aa), FAD binding-2(66-389aa) and FAD binding-3(64-412aa). One of the domains DAO belongs to the class of FAD flavoenzymes involved in the oxidation of basic and neutral D-amino acids into keto acids. Squalene epoxidase (SE) domain is found in taxonomically diverse groups of eukaryotes and bacteria while as FAD binding domain is involved in FAD binding with various enzymes using

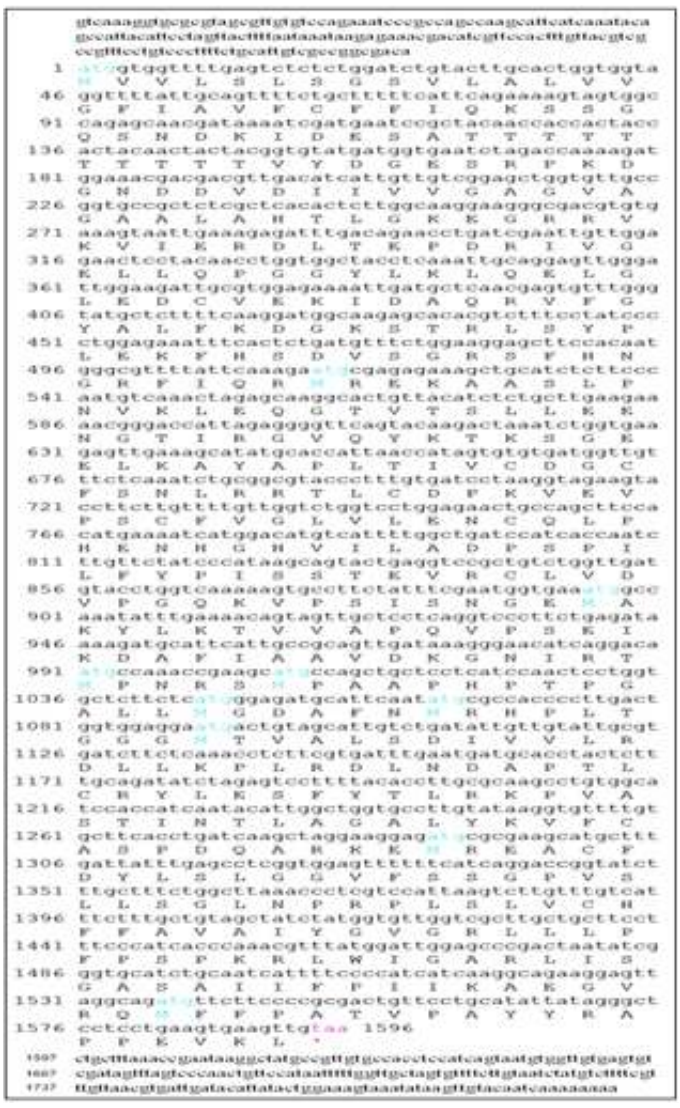


Fig. 2 Nucleotide and the deduced amino acid sequence of *WsSQE* from *W. somnifera.* The ATG start codon at position 1 and the TAA stop codon at position 1596 are highlighted

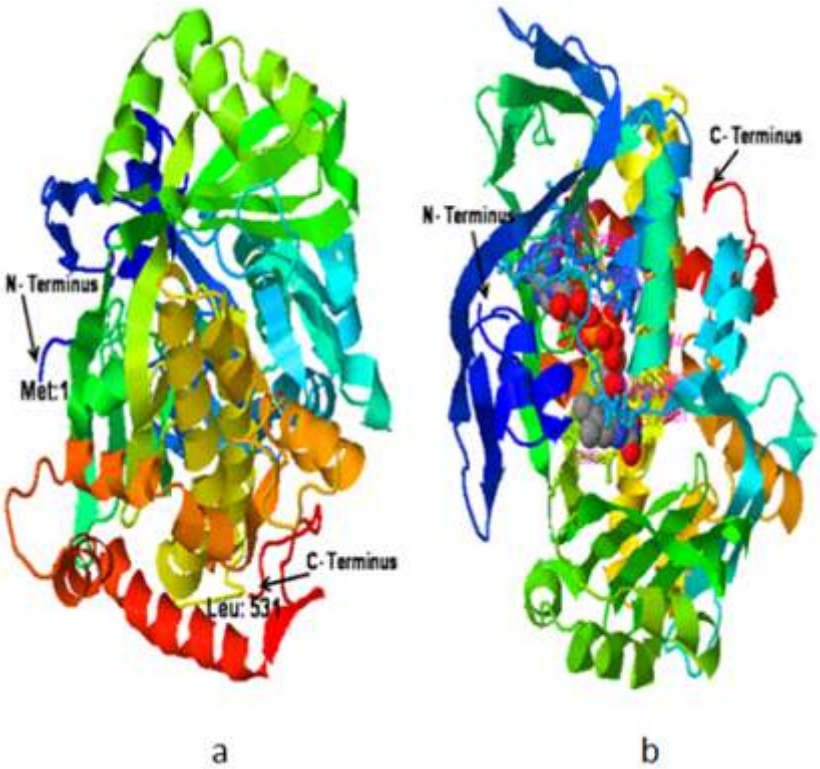


Fig.3 Predicted 3-D structure (a) and substrate binding site (b) of *WsSQE* deduced by I-TASSER homology- based modeling using *Pseudomonas aeruginosa* FAD dependent monooxygenase (pdb2x3n) as template.

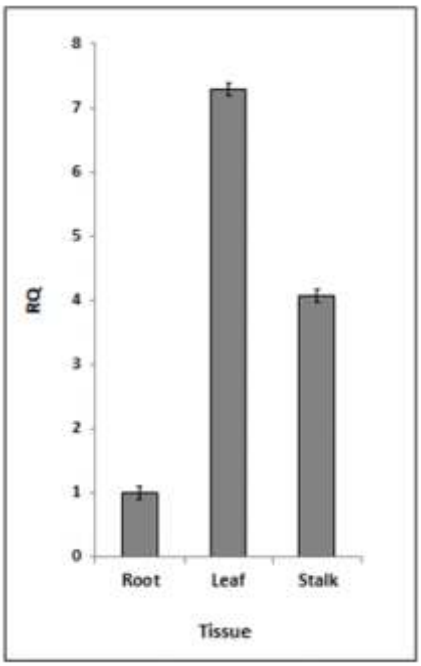


Fig.4 Comparative tissue specific expression of WsSQE

FAD as cofactor and are responsible for many catalytic properties in the living system. The secondary structure analysis of *WsSQE* was done by SOPMA (*http://expasy.org/tools/SOPMA*). Results showed that predicted *Ws*SQEprotein consists of 36.35% alpha helices, 19.02% extended strands, 38.79% random coil, and 5.84% beta turns. Alpha helices and random coils were most predominant, while beta turns were least abundant structures in the predicted *WsSQE* protein.

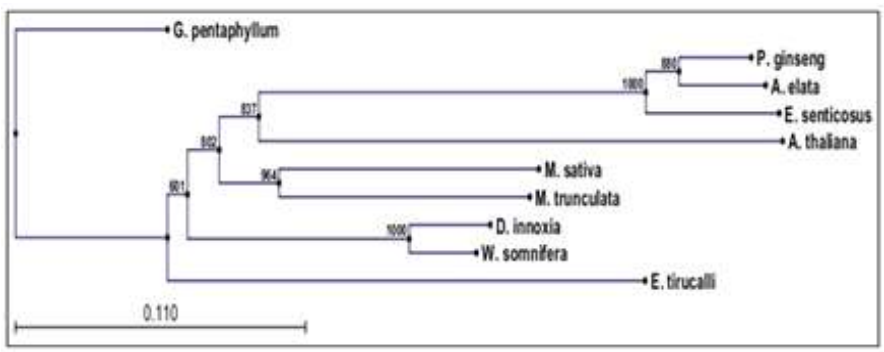


Fig. 5 Phylogenetic tree of squalene epoxidase proteins sequences of various plants: *W.somnifera* (GenBankaccession number GU574803), *D.innoxia* (GenBank Acc. No. AAY22200), *G.pentaphyllum* (GenBank Acc.No.ACQ90301), *N. sativa* (ACJO533), *A. elata* (GenBank Acc. No.ADC32655), *P. ginseng* (GenBankAcc. No. BAD15330), *E. tirucalli* (GenBank Acc. No. BAF79915).

The three dimensional structure of *WsSQE* (Fig. 5) was predicted by I-TASSER web server (http://zhang.bioinformatics.ku.edu/I-TASSER). The *Pseudomonas aeruginosa* FAD dependent monooxygenase was used as template to carry out homology based structural modeling FAD ligand exhibited binding at various residues in this model having similarity (BS > 1.1) with binding sites of *WsSQE* protein sequence.

Comparative tissue specific expression

Relative transcript analysis of *WsSQE* was performed by using equal amount of RNA sampled from different tissues (leaves, stalks and roots) of *W. somnifera* by using quantitativePCR. The expression levels in the all the tissues were normalized against the normal transcript levels of actin, which was taken as a control in this experiment. Real-time PCR results revealed that *WsSQE* transcripts were relatively abundant in leaf tissue, followed by stalk and least in the root tissue (Fig. 6). The high expression of *WsSQE* in leaves corroborates well with the higher accumulation of withanolides in leaves as reported in earlier studies.

Bacterial expression of *WsSQE*

The entire protein coding cDNA of *WsSQE* was cloned into expression vector pGEX-4T-2, containing a functionally hybrid *tac* promoter and glutathione S-transferase (GST; 26 kDa) as a fusion tag to facilitate subsequent purification of the recombinant *WsSQE* protein. The pGEX-*WsSQE* construct was sequenced and transformed into *E.coli* BL-21 cells the resultant recombinant *WsSQE* fusion protein of approximate size 85.1 kDa (Fig. 7) was observed on10% SDS gel.

Promoter isolation *of WsSQE*

Using the PLANT CARE and PLACE web tools several putative *cis*-regulatory elements were deciphered from the promoter sequence of *WsSQE* (Fig. 8). TATA box sequence elements required for the critical and precise transcription initiation were found at positions 96 (-), 5' upstream region of the of the start codon. CAAT BOX sequences responsible for the tissue specific promoter activity were found at numerous positions 226 (-), 299 (-), 497 (-), 144 (-), 511 (+), 149 (+). Calcium responsive cis-element ABRERATCAL was found at 240 (+) position. ACGTATERD sequence element required for etiolation based expression of *erd1* was identifiedat469(+) position. Conserved sequence AMYBOX1 found in 5'-upstream region of alpha-amylase gene of rice, wheat, barley was present at 463 (-) positions in promoter. BIHD1OS binding site of OsBIHD1,

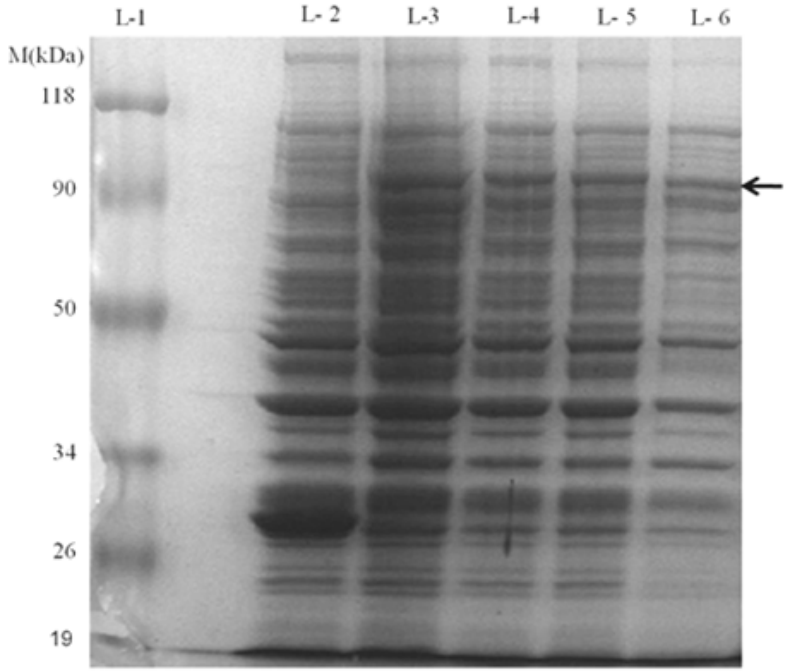


Fig 6 Heterologous protein expression in *E. coli* with IPTG induction (1mM) at 37°C for 2, 4, 6, 8 h. Protein Molecular Weight Marker (MW) (L1), *E.coli* harboring empty vector (L2), *E.coli* harboring pGEX-SQE construct induced by IPTG (1mM) at 2 h (L3), 4 h (L4), 6 h (L5) and 8 h (L6).

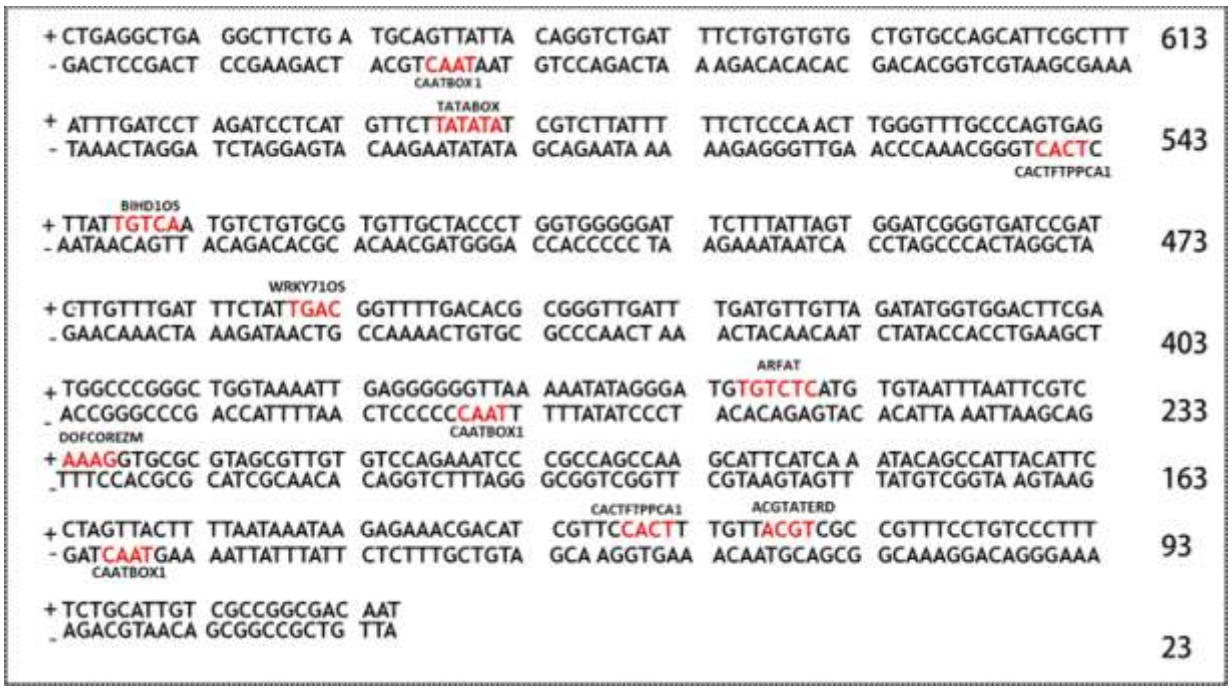


Fig. 7 The putative *cis*-regulatory element present in the promoter region of the *WsSQE*. A 580 bp fragment was amplified from the genome walker library using *EcoRV* restriction enzyme. The key sequence elements TATABOX, CAATBOX1, ABRERATCAL, ARR1AT are highlighted

positions. ARR1ATand ARR1 transcription factors for genes, which are involved in cytokinine response, were located at 180 (+), 138 (+), 218 (+), 249 (+) homeodomain transcription factor were found at 146 (+), 237 (-). CACTFTPPCA1 460 (+), 427 (+), 136 (-), 192 (-) tetra nucleotide (CACT) is a key component related to the photosynthetic process. DOFCOREZM 352 (+), 185 (-), 429 (-), 462 (-), 488 (-) transcription factors are associated with the expression of multiple genes involved in carbon metabolism in maize .

Some important phytohormone

induced regulatory elements like ABREOSRAB21 469 (+), ARFAT 327 (+), ASF1MOTIFCAMV 228 (+), 348 (-), WRKY71OS 228 (+) were also found in the promoter region indicating active transcriptional regions in response to various phytohormones. The key promoter elements and their putative functions are listed in the Table 2.

3.12 Cloning and heterologous expression of CYP76 and CYP98 hydroxylases from *Withania somnifera*

Satiander Rana, Rekha S. Dhar, Surrinder K. Lattoo and Ram Vishwakarma

Cytochrome P450 monoxygenase belongs to largest gene superfamily. They catalyze diverse array of reactions like epoxidation, hydroxylation, and alkylation. Mainly they are involved in secondary metabolite biosynthesis. Most cytochrome P450 enzyme systems consist of two proteins, usually both anchored in the endoplasmic reticulum, the NADPH:cytochrome P450 reductase and the cytochrome P450 itself. The latter confers binding and activation of molecular oxygen, as well as substrate specificity and catalytic conversion. P450s catalyze rate-limiting and sometimes complex chemical reactions in all plant-specific metabolic pathways, like phenolic and lipid metabolism, biosynthesis of isoprenoids, alkaloids and other amino acid derived compounds.

W. somnifera is a rich source of pharmacologically active steroidal lactones known as withanolides. The downstream pathway responsible for their biogenesis is unknown. To identify the key genes involved in withanolide biosynthesis we have successfully cloned and expressed two P450 monoygenase namely *Ws*CYP76 (Genbank ACC. No. HM06990) and *Ws*CYP98 (Genbank ACC. No. HM06610) having open reading frame of 1548 bp and 1536 bp, containing 515aa and 511aa respectively.

For heterologous expression, the restriction sites were added to their respective ends and both expression vector and coding domains were digested with the restriction enzymes. Digested vector and insert were ligated using T4 DNA ligase at 16° C overnight. The ligated products were then transformed and expressed in *E. coli* (BL21).

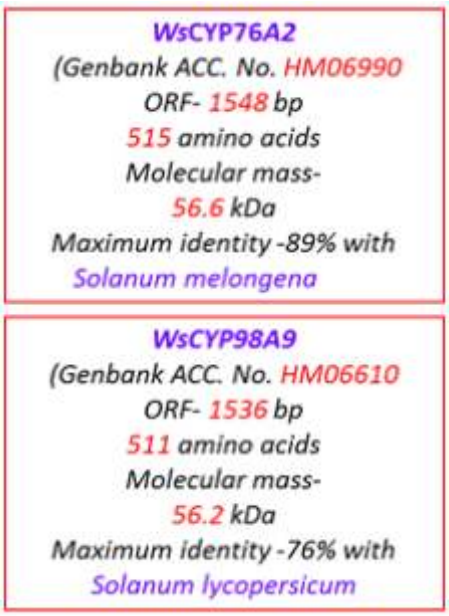


Fig. 1 Heterologous expression of *Ws*CYP76 and *Ws*CYP98 in*E coli* (BL21) using 0.8 mM IPTG. In different lanes. Lane M: Marker, C: Control and 2h, 4h & 6h: Samples harvested after two four and six hours.

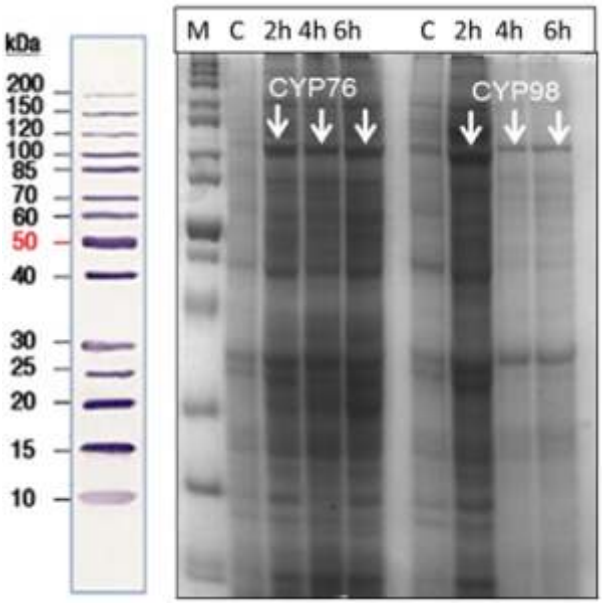
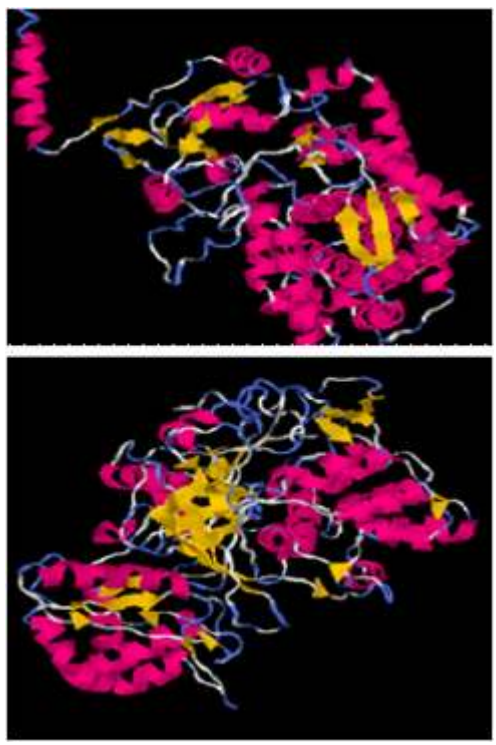


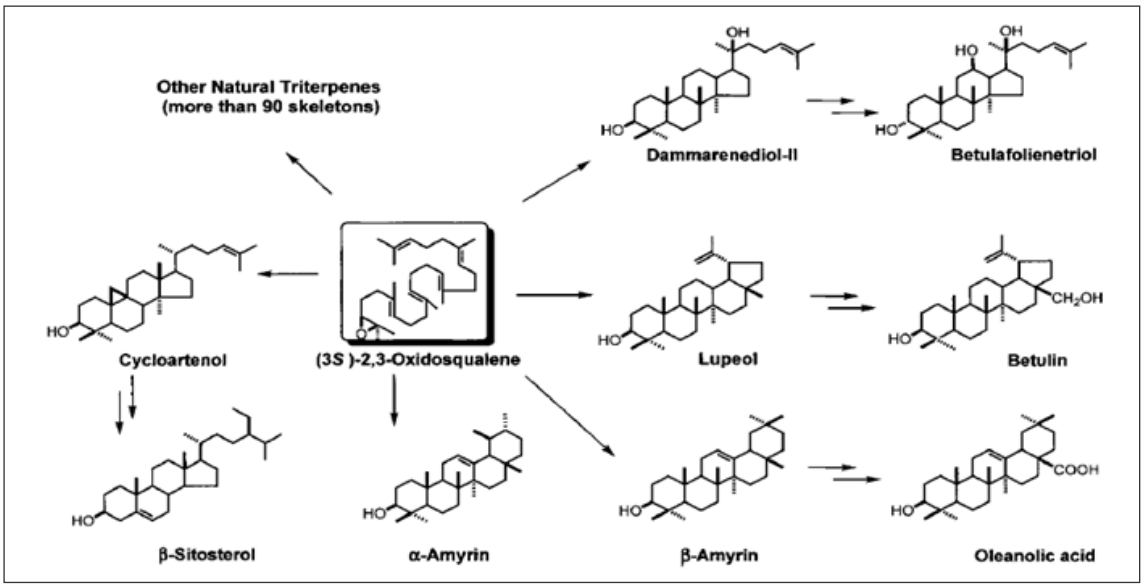
Fig. 2 Predicted 3 dimensional protein structures of *Ws*CYP76 and *Ws*CYP 98 on the basis of homology modeling using online webserver I-TASSER



3.13 Molecular cloning, heterologous expression, quantitative real-time expression analysis of β-amyrin synthase and Lupeol synthase from *Withania somnifera*

Niha Dhar, Satiander Rana, Nasheeman Ashraf, Rekha S. Dhar, Surrinder K. Lattoo and Ram Vishwakarma

Triterpenoids are a very diverse group of natural products with wide distribution and particularly high chemical diversity in plants. They include compounds such as withanolides, lupeol, beta-amyrin, betulinic acid, the avenacins, and glycyrrhizin, which have important biological functions and medicinal properties. The biosynthetic pathway toward triterpenoids proceeds by cyclization of 2, 3-epoxysqualene to different triterpenoid products involving various members of oxidosqualene cyclases (OSC) gene family. Similarly in *Withania somnifera* this step is situated at the critical branching point for phytosterol/withanolide and triterpenoid biosynthesis as shown in figure 1. Sequence generation, analysis and functional characterization of various members of OSC family can open gateways for engineering the desired triterpenoid pathway. As a step towards this we have cloned and characterized β- amyrin synthase (*WsBS*) and Lupeol synthase (*WsLS*) of OSC family from *Withania* somnifera.



Oxidosqualene cyclization in higher plants

*Ws*BS &*Ws*LS were amplified using degenerate primers based on the conserved regions of reported OSCs. PCR fragments were cloned into pGEM®-T vector (Promega) and sequenced. 5´ & 3´ RACE were performed using FirstChoice ® RLM-RACEKit and the sequence generated was used for designing full length primers. Open reading frames of WsBS system using *pGEX* bacterial expression vector and BL21 strain of E.coli . Further the expressed proteins were purified using GST sepharose beads (GE healthcare).

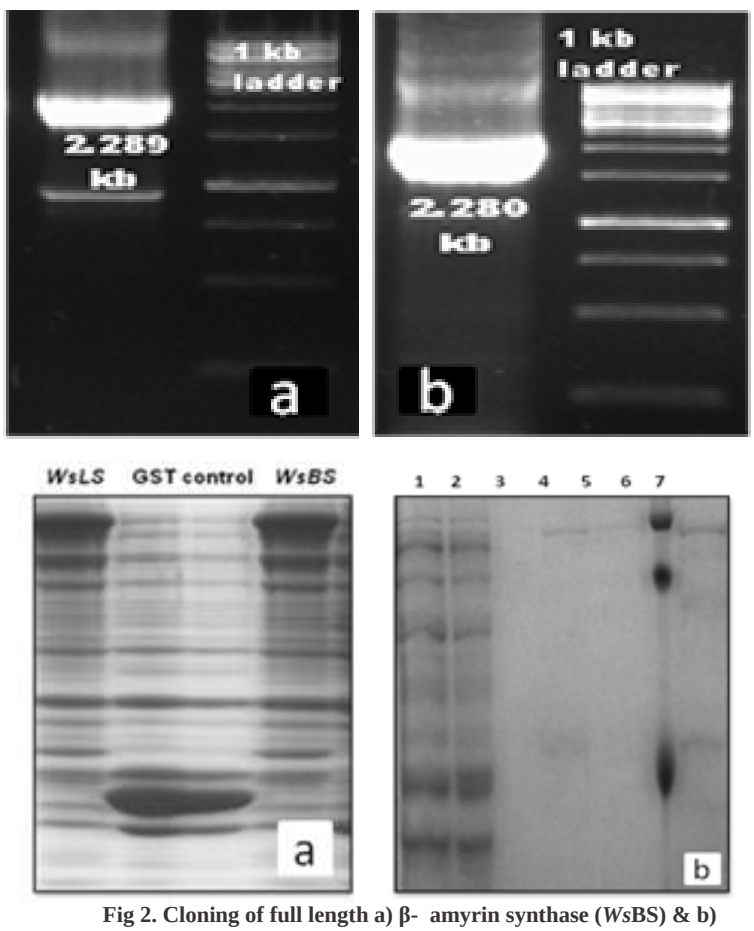


Fig 2. Cloning of full length a) β- amyrin synthase (*Ws*BS) & b) Lupeol synthase

Fig 3. Heterologous expression of a) *Ws*BS &*Ws*LS and CBB stained image of b) purified WsBS & WsLS ON 12.5 % SDS

To account for relative transcript level of the two cloned genes in different tissues, a quantitative PCR was performed. Both the genes showed significantly high expression in roots

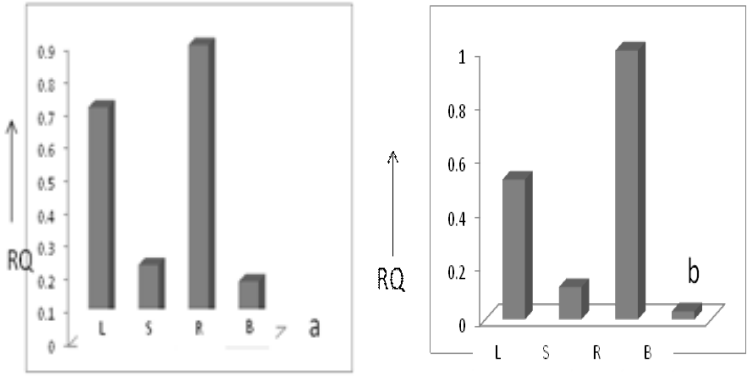


Fig 4. Tissue specific real time expression profile of a) *Ws*BS & b) *Ws*LS

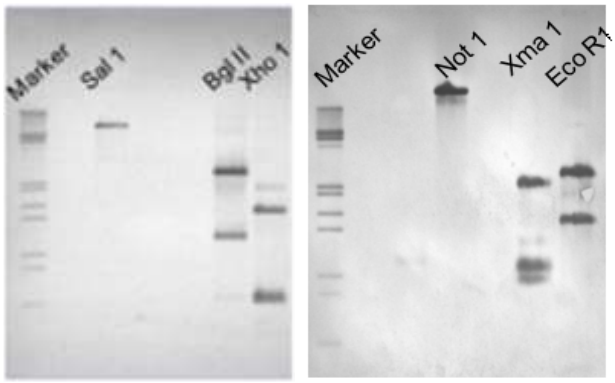


Fig 5. Copy number analysis of a) WsBS & b) WsLS using DIG DNA Labeling and Detection Kit (Roche)

(R) followed by leaves (L), stalk (S) and berries (B). Further to probe the copy number of *WsBS* and WsLS, southern blotting was done using *DIG* DNA Labeling and Detection Kit (Roche) which illustrated both genes to possess a single copy number in *W.somnifera.*

3.14 An *in vitro* approach to molecular infidelity yields 12-deoxywithastramonolide rich chemovariant in *Withania somnifera* (L.) Dunal - its molecular cytogenetic analysis and significance as a chemotypic resource

Satiander Rana, Niha Dhar, Shabnam Khan, Rekha S. Dhar, Prabhu Dutt and Surrinder K. Lattoo

An *in vitro* approach is a viable alternative to induce epigenetic and genetic changes in crop species. Plant characteristics such as tolerance to various types of stresses, disease resistance, and qualitative or quantitative improvement in yield attributes have been achieved in variety of plants. The present investigation validates the applicability of an *in vitro* strategy to induce somaclonal variation in *W. somnifera* which manifested in the form of enhanced levels of WS-12D.

Six weeks after culture initiation, the morphogenetic response of *W. somnifera* explants on media supplemented with Kn in combination with various concentrations of 2,4-D, IBA, or NAA was recorded. Kn/2,4-D and Kn /NAA combinations, at varying concentrations of the auxins (0.51.5 mg l^{-1}), resulted in pale-white friable callus with sparse shoot differentiation and white-green friable callus with a moderate number of shoots, respectively. The percentage of callus formation ranged from 2452% on Kn/2,4-D combinations. On the other hand, callus induction on Kn/NAA combinations ranged from 5260%, with a mean shoot length of 4.2cm and an average of 5 shoots per explant.

However, the combination of Kn and IBA was found to be most effective in terms of both callus induction and shoot regeneration (Fig. 1*a, b*), and showed a better response compared to other hormonal supplements reported for *W. somnifera*. Within 6 wk after inoculation on MS medium containing 2 mg l^{-1} Kn and 1 mg l^{-1} IBA, profuse pale white-green

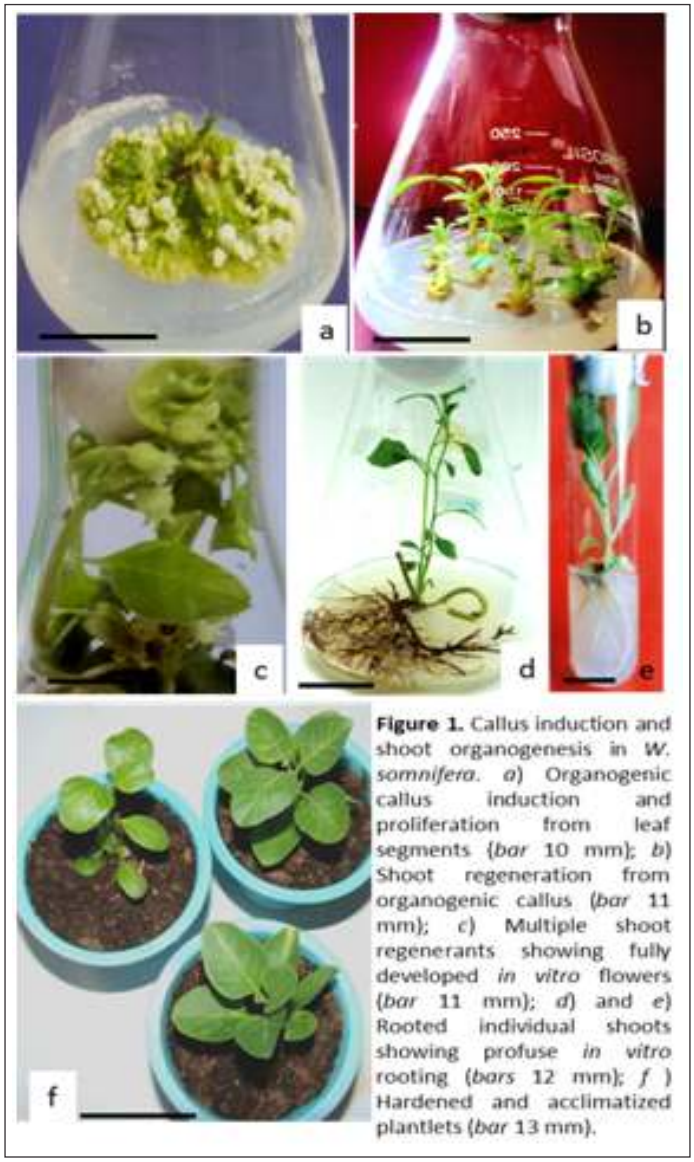


Figure 1. Callus induction and shoot organogenesis in *W. somnifera*. *a*) Organogenic callus induction and proliferation from leaf segments (*bar* 10 mm); *b*) Shoot regeneration from organogenic callus (*bar* 11 mm); *c*) Multiple shoot regenerants showing fully developed *in vitro* flowers (*bar* 11 mm); *d*) and *e*) Rooted individual shoots showing profuse *in vitro* rooting (*bars* 12 mm); *f*) Hardened and acclimatized plantlets (*bar* 13 mm).

nodular callus was successfully induced (Fig. 1*a*). The overall percentage of callus formation ranged from 7696% at varying concentration of IBA (0.5–1.5 mg l^{-1}). Multiple shoot regeneration waspreceded by shoot bud differentiation. A significantly higher number of shoots per explant (15.80 ± 0.73) with an average shoot length of 6.90 ± 0.47 cm was achieved with 2 mg l^{-1} Kn and 1 mg l^{-1} IBA.

Interestingly, plantlets obtained with Kn and IBA flowered profusely *in vitro* (Fig.1*c*). These flowers were fully functional, producing yellow berries with viable seeds. Shoots transferred to rooting medium exhibited induction of vigorous tapering roots (Fig. 1*d–e*). Optimum root induction was observed with 0.75 mg l^{-1} IAA. Root initiation started 3 wk after transfer onto rooting medium. Rooting percentage with different concentrations of IAA ranged from 44–92%. Following hardening procedures, 5 wk old hardened plants (Fig. 1*f*) were successfully transferred to field conditions, with a survival rate of about 86%. *Chemoprofiling.*In total, 54 somaclones were successfully established under field conditions. These were free of any noticeable phenotypic variability compared to the donor mother plant. Almost all of the tested regenerated plants established under field conditions showed a detectable, moderate variation in the chemical constituents. One somaclone, designated WS-R-1, showed a significant accumulation of WS-12D amounting to 0.516 %, compared to 0.002% in the mother plant. The HPLC chemoprofile of the mother plant indicated the absence of WSC-O, while WS-R-1 was devoid of two constituents (WS-2 and WSC-O; Fig. 2*b–c*).Other constituents namely WS-1, WS-2, WS-3, WSC, and WSG-3 showed a decline when compared with the mother plant. The major reduction was in WS-3 which accumulated at 0.136% in the somaclone as compared to 0.755% in the mother plant. The comparative chemoprofiles were consistent for two years of growth under uniform cultivation conditions. *In vitro* stress coupled with regeneration via a callus phase is a proven approach to trigger molecular instability. Nevertheless, the

Fig. 2

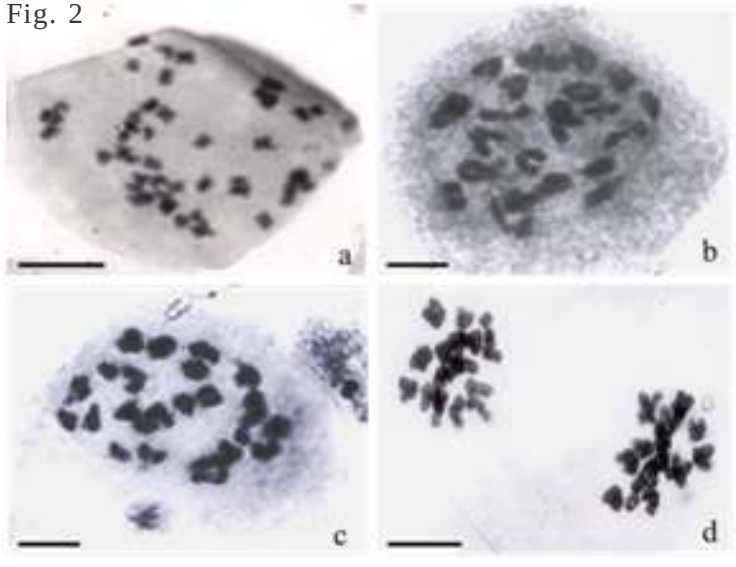


Figure 2.HPLC profile of withanolides in *W. somnifera*. *a*) Standard withanolide markers; *b*) Mother plant leaf extract; *c*) Somaclonal variant leaf extract. Withanolide-A (WS-1), withanone (WS-2), withaferin-A (WS-3), 12-deoxywithastramonolide (WS-12D), withastramonolide (WSC), 27-hydroxywithanone, (WSC-O) , withanoside (WSG-3).

Fig. 3

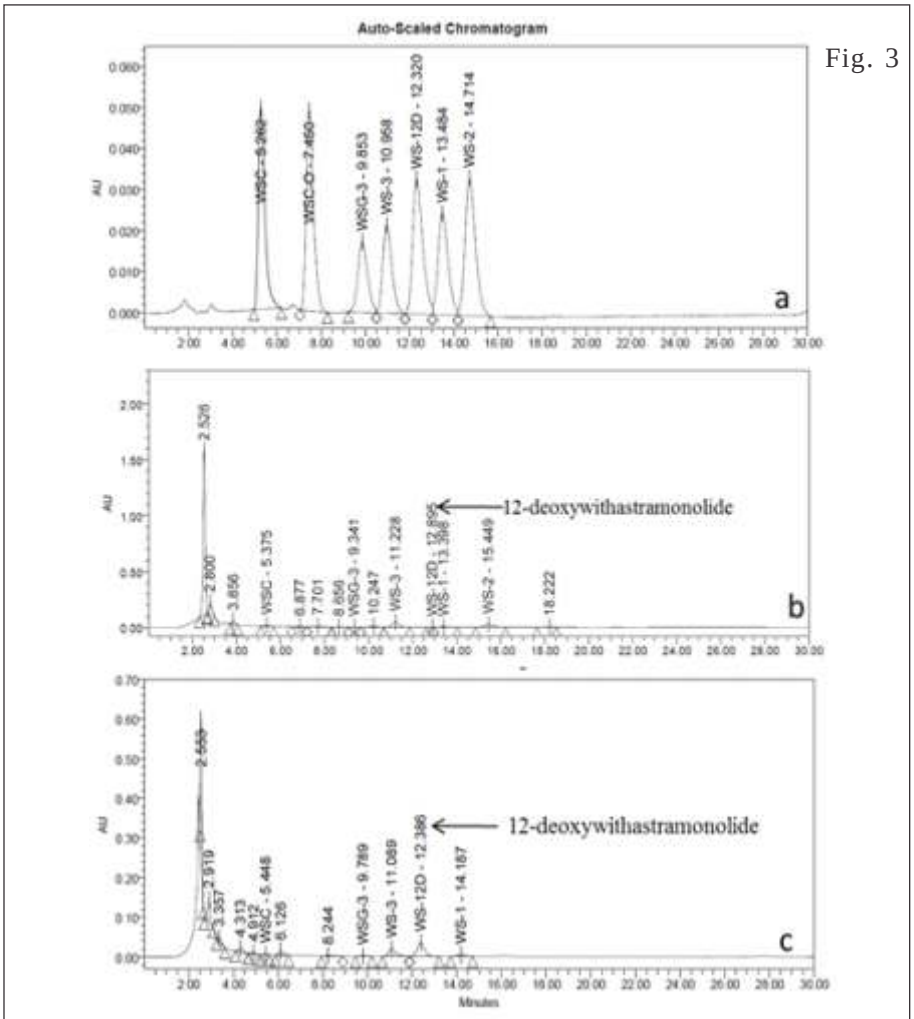


Figure 3.*a*) Somatic metaphase spread showing 48 chromosomes, *b*) Diakinesis, *c*) Meiotic metaphase-I presenting 24^{II}, *d*) Late anaphase-I showing normal 24:24 disjunction. *Bars* 10 µm.

underlying changes in WS-R-1 which resulted in overproduction of WS-12D and reduced accumulation of WS-3 requires molecular dissection. Biogenesis of withanolides is complex as various steps involved in biosynthesis of key withanolides are yet to be fully elucidated. Different chemotypic varieties provide an important resource for deciphering the biosynthetic pathway of withanolides. These metabolites are synthesized through the isoprenoid pathway, probably via both mevalonate and non-mevalonate pathways, wherein 24-methylene cholesterol is the first branching point towards the biosynthesis of different withanolides through a series of desaturation, hydroxylation, cyclization, chain elongation and glycosylation reactions.

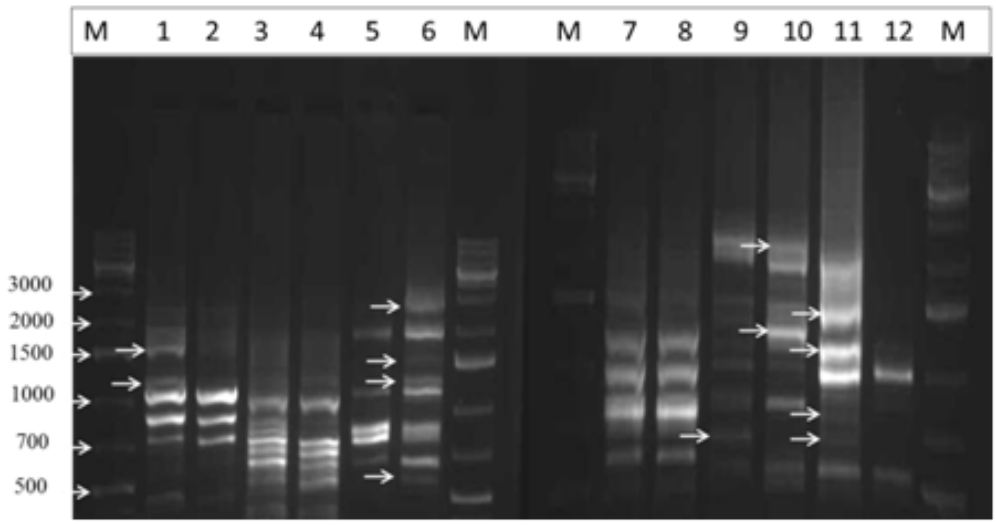


Figure 4. DNA fingerprints obtained with primers S-109 (*lanes1, 2*), S-144 (*lanes 3, 4*), S-164 (*lanes 5, 6*), S-361 (*lanes 7, 8*), S-373 (*lanes 9, 10*) and S-377 (*lanes 11, 12*). Odd lanes (*1, 3, 5, 7, 9,* and *11*) represent mother plant; even lanes (*2, 4, 6, 8, 10,* and *12*) represent W-1-R somaclone. *M*, Molecular weight marker (1 kb DNA ladder). Polymorphic bands are indicated by *arrows.*

*Cytological analysis.*The diploid chromosome number for *W. somnifera* was found to be 2N = 48 (Fig. 3*a*). There were no anomalies in gross chromosome structure and organization for any of the regenerated plants. Meiosis was normal with perfect chromosome pairing and 24 bivalents at diakinesis and metaphase-I (Fig. 3*b–c*). Chromosome segregation was normal with 24:24 disjunction at anaphase-I of meiosis (Fig. 3*d*). Normal meiosis resulted in high pollen fertility (89%) and average seed set per fruit was 78.5%.

*RAPD analysis.*Genetic fidelity of *in vitro* regenerated plants has immense practical utility and commercial applications. Keeping this perspective in mind, the WS-R-1 somaclone, and its explant donor mother plant were subjected to comparative RAPD analysis. Ten random RAPD primers were tested, from which 6 gave reproducible results.

Among these 6, 2 primers namely, S-164 and S-373 gave monomorphic bands and the remaining 4 showed altered banding patterns (Fig. 4). A total of 82 band positions were recorded which were generated by 6 primers ranging from 600 – 2800 bp in size. DNA from the somaclone gave five unique amplified bands of 600, 700, 1400, 1500, and 2800 bp in size.Appearance or disappearance of RAPD bands can be due to a single base change at the primer annealing site. Tissue culture conditions may have stimulated various genetic changes in the regenerated plants. Thus, molecular changes can reflect stable changes in the genome which can be used to breed for stable, desirable phenotypes.

To our knowledge, this is the first report of somaclonal variation in *W. somnifera* providing a stable chemovariant with increased WS-12D content. This somaclone was evaluated for two years to confirm genetic and chemical stability. This stable WS-12D rich somaclone provides a new addition to the already existing germplasm of 27 accessions procured from different eco-geographic regions of India. A species-rich germplasm resource base and an efficient breeding system are prerequisites for optimum genetic amelioration and effective utilization of allelic and chemotypic variability. *W. somnifera* displays a versatile sexual mechanism as its hermaphroditic flowers practice mixed mating which presents an opportunity to receive potential benefits from both out-crossing as well as self-fertilizing events. Coupled with efficient mating, its meiotic system also guarantees sufficient release of variability through genetic recombination.

This study thus supports the feasibility of an *in vitro* strategy for the recovery of hyper-productive chemotypes of pharmacological significance.Both conceptually and empirically, it also widens the genetic resource base for manipulative hybridization for quantitative chemotypic novelty in *W. somnifera*.

3.15 Identification of two divergent isofoms of UDP-Glycosyltransferases from *Picrorhiza kurroa*

Wajid Waheed, Rekha S. Dhar, Surrinder K. Lattoo and Ram Vishwakarma

Glycosyltransferases (GTs) constitute a superfamily of enzymes that catalyze conjugation of carbohydrate moieties to oligo/polysaccharides, proteins, lipids, terpenoids, flavanoids, alkaloids and other small molecules. Over 12,000 sequences encoding GTs are available in CAZy database (http://afmb.cnrs-mrs.fr/CAZY). The superfamily consists of 78 families based on sequences, signature motifs, stereochemistry of glyco-linkages, substrate specificity, nature and range. GTs that use UDP-activated sugars as donors and various types of small molecules as acceptors are called UDP-glycosyltransferases (UGTs) and represent family 1 GTs. In higher plants, UGT catalyzed glycosylation constitutes a prominent terminal modification in the biosynthesis of secondary metabolites and generates diverse natural glycosides (Bowles, D. *et al* 2006). The UGTs possess substrate specificity based on regioselective recognition of chemical sub-structures. Biological functions of glycosylations in plants include storage, inter and intracellular transport of metabolites, regulation of homeostasis of hormones, etc. UGTs glycosylating at –OH, –COOH, –NH2, –SH and C–C groups in secondary metabolites have been reported. In an endeavor towards metabolic engineering in *Picrorhiza kurroa*, we have successfully isolated full length cDNAs of two divergent isoforms of UDP-Glycosyltransferases.

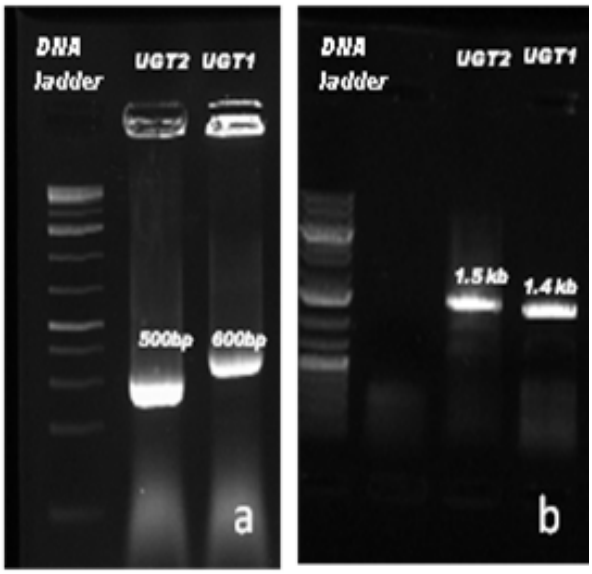


Fig. 1 Amplification of two divergent isoforms of UDP glycosyltransferases (a & b) from *P. kurroa*

Open reading frame of *PkUGT1* and

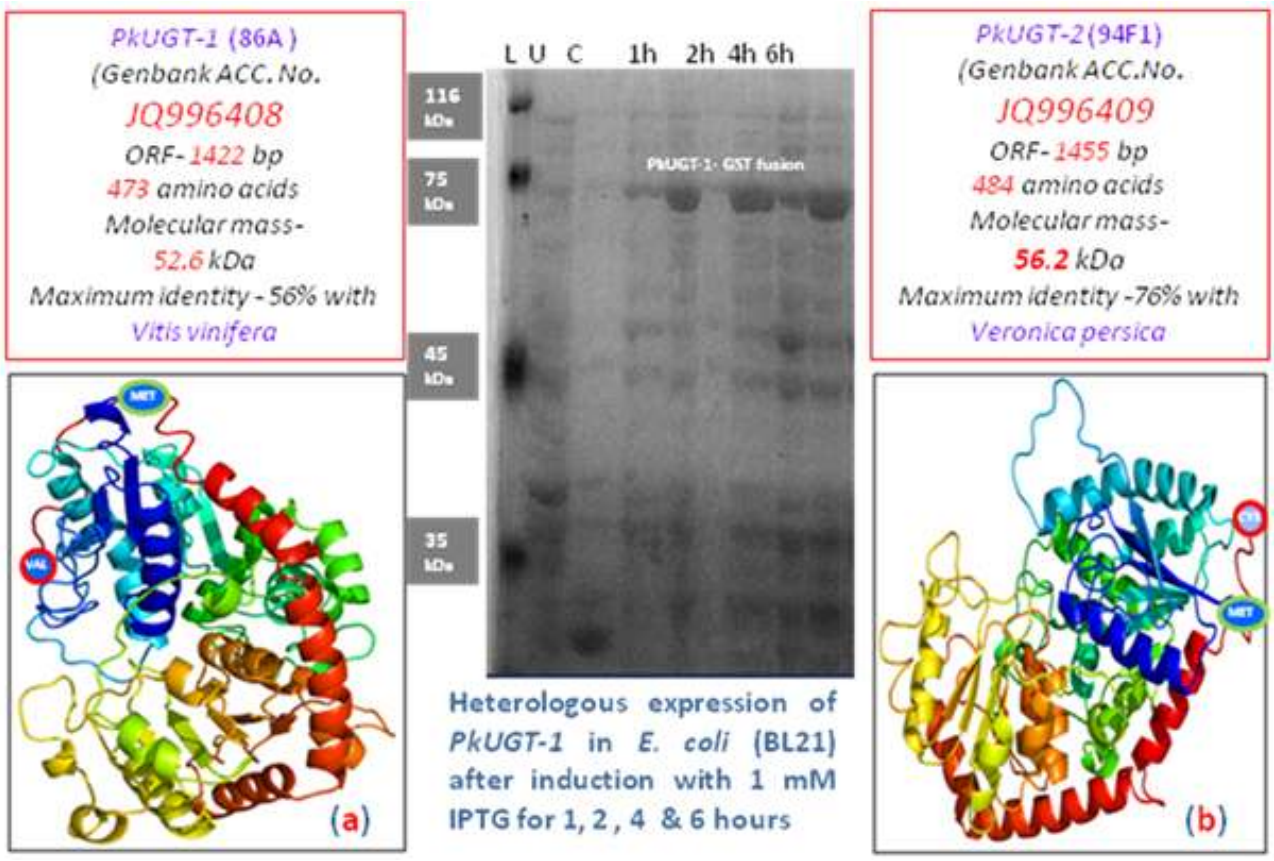


Fig. 2 Predicted 3D structure of *PkUGT1* (a) and *PkUGT2* (b) constructed by homology modeling software- PHYRE 2 using crystal structure of flavonoid 3-o-glucosyltransferase (c3hbjA) as template.

PkUGT2 were 1422 and 1455 bp long encoding 473 and 484 amino acids respectively. ClustalW2 alignment of the deduced amino acid sequences showed maximum similarity with the orthologs from *Vitis vinifera* (50%- *PkUGT1*) and *Vernoica persica* (53%- *PkUGT2*). Predicted molecular mass of *PkUGT1* and *PkUGT2* were 52.3kDA and 55kDA respectively. Predicted pI was 4.92 and 5.16 respectively.

Full length genes of *PkUGT1* and *PkUGT2* were cloned into pGEX-4T2 vector and expressed in E. Coli (BL21) and the recombinant proteins obtained are being further studied for functional validation

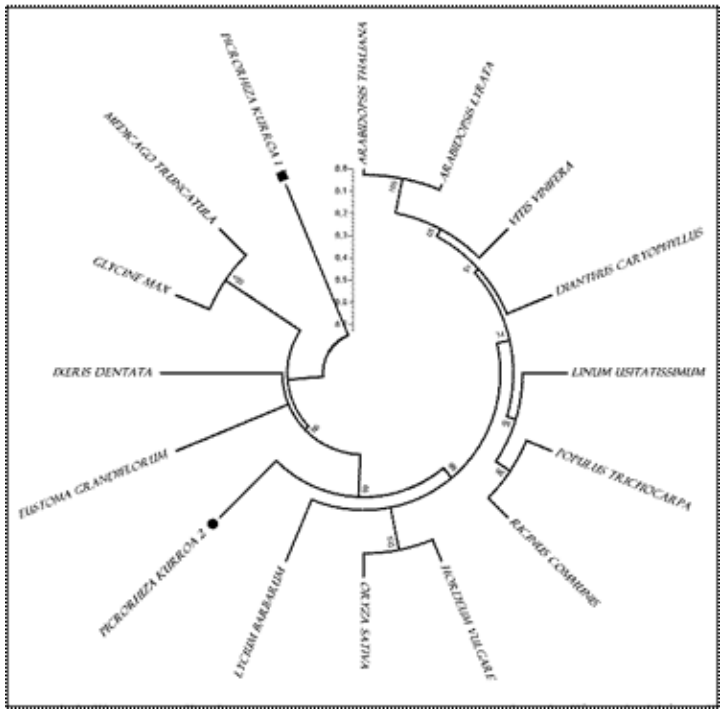


Fig. 3 Neighbour joining phylogenetic tree constructed from the deduced amino acid sequences of *PkUGT1* and *PkUGT 2* isolated from *P. kurroa* and UGTs from other plant species using MEGA 5.05

4. ANTI-CANCER THERAPEUTICS

Potentiation of the antitumor effect of 11-keto-β-boswellic acid by its 3-α-hexanoyloxy derivative

Gousia Chashoo, Shashank K. Singh, Dilip M. Mondhe, Parduman R. Sharma, Samar S. Andotra, Bhahwal.A. Shah, Subhash C. Taneja and Ajit K. Saxena

Abstract

We recently discovered that a propionyloxy derivative of 11-keto-β-boswellic acid (PKBA) showed better anticancer potential than other boswellic acids including AKBA, encompassing the importance of acyl group at the 3-α-hydroxy position of KBA. In continuation of our previous work, other higher derivatives (with increasing alkoxy chain length at 3-α-hydroxy position) including butyryloxy (BKBA) and hexanoyloxy (HKBA) derivatives of KBA were synthesized. The respective IC_{50} values of BKBA and HKBA in HL-60 cells were found to be 7.7 and 4.5 µg/ml. IC_{50} value of HKBA was comparatively lower than that of BKBA, and further lower than that of the previously reported derivative (PKBA, IC_{50} 8.7 µg/ml). In order to compare the anticancer potential of HKBA with PKBA, detailed *in vitro* pro-apoptotic and *in vivo* anticancer studies were carried out. The induction of apoptosis by HKBA was measured using various parameters including fluorescence and scanning electron microscopy, DNA fragmentation and Annexin V-FITC binding. The extent of DNA damage was measured using neutral comet assay. HKBA was further evaluated for its effect on DNA cell cycle and mitochondria where it was found to arrest cells in G_2/M phase and also induced loss of mitochondrial membrane potential. These events were associated with increased expression of cytosolic cytochrome c and cleavage of PARP. Target based studies showed that HKBA inhibited the enzymatic activity of topoisomerases I and II at low doses than that of PKBA. *In vivo* studies also revealed a low dose inhibitory effect of HKBA on ascitic and solid murine tumor models.

Results

In vitro anti proliferation activity in human leukemia cell lines

The cell growth inhibition of test compound in human leukemia cell lines was tested by MTT assay as described earlier. HKBA inhibited the cell growth in dose dependant pattern and the IC_{50} value of 4.5 µg/ml in HL-60 and 6 µg/ml in Molt-4 cancer cell lines after 48 h of treatment was observed (Fig.1). The IC_{50} values of HKBA were found to be lower than that of BKBA (HL-60, 7.7 µg/ml and Molt-4, 9 µg/ml) and previously reported PKBA (HL-60, 8.7 µg/ml and Molt-4, 9.5 µg/ml).

In vivo antitumor activity

In vivo anticancer studies of HKBA revealed that tumor growth was

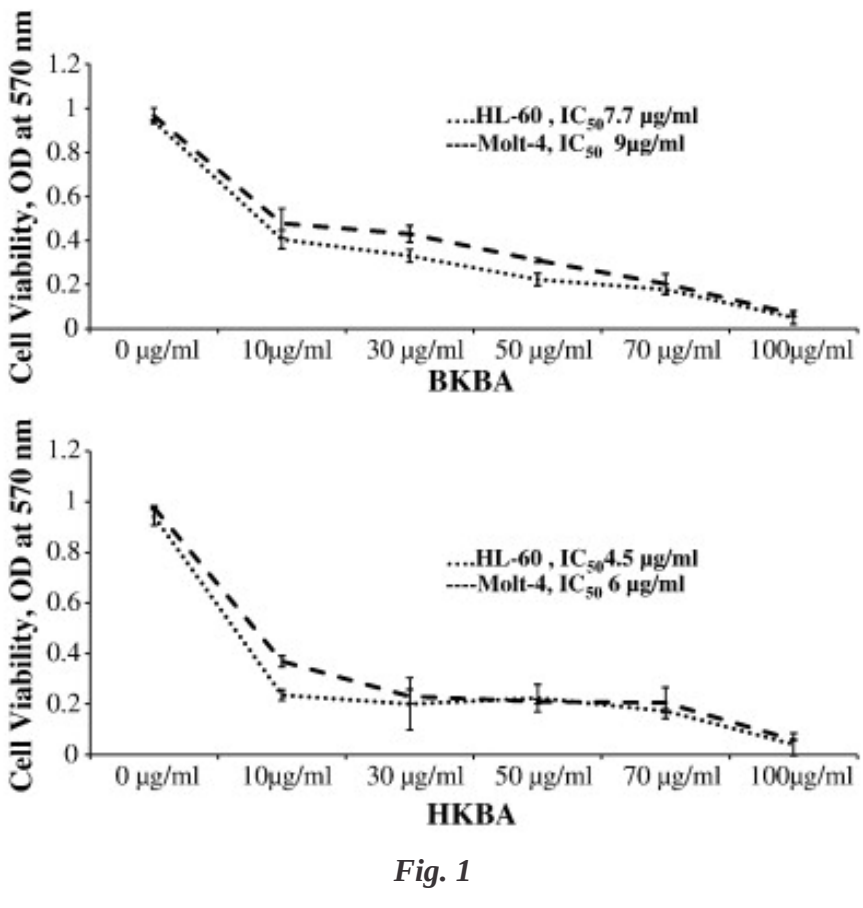


Fig. 1

inhibited dose dependently in the mice bearing ascitic (EAC) and solid (EAT and S-180) tumors. At intraperitoneal doses of 50 and 60 mg/kg b.wt.the growth of Ehrlich ascitic carcinoma (EAC) was inhibited by 47.54% and 67.29% respectively. In case of solid tumors, HKBA showed a growth inhibition of 35.65% and 50.64% at 50 and 60 mg/kg b.wt. respectively in EAT model and 38.35% and 54.36% at the respective doses in S-180 model. PKBA on the other hand was found to show the same effect in both ascitic and solid tumor models at 150 mg/kg b.wt. (Chashoo et al., 2011). Tumor growth inhibition by 5-FU used as positive control was found to be 48.11% and 46.75% at 22 mg/kg b.wt. in ET and S-180 respectively (_Table1).

Table 1. The *in-vivo* anti-cancer studies of HKBA indicate a dose dependent inhibition of different tumor models in Swiss albino mice. At 60 mg/kg body wt. HKBA showed 67.29% growth inhibition of ascitic tumor model namely Ehrlich ascites carcinoma (EAC). The growth inhibition induced in solid tumor models namely Ehrlich tumor (ET) and Sarcoma-180 (S-180) at 50 and 60 mg/kg was found to be 35.65 and 50.64% respectively for ET and 38.35 and 54.36% respectively for S-180. The respective growth inhibition caused by 5-Flurouracil was 48.11 and 46.75% in ET and S-180 tumor models at 20 and 22 mg/kg respectively.

Sample	Dose (mg/kg i.p.)	Animals/mortality	Tumor volume (ml)	Cell count (10^7)	% Tumor growth inhibition
A). *In vivo* anticancer activity of HKBA against Ehrlich ascites carcinoma					
Control	NS	10/0		286.95 ± 3.26	
HKBA	50	7/0	4.79 ± 0.22	150.52 ± 5.60	47.54 ± 0.34[a]
	60	7/0	3.14 ± 0.14[a]	93.84 ± 0.51[a]	67.29 ± 0.48[b]
5 FU	20	7/0	0.92 ± 1.02[a]	15.32 ± 1.3[a]	94.66 ± 0.32[b]
Sample	Dose (mg/kg i.p.)	Animals/mortality	Body weight (g)	Tumor weight (mg)	% Tumor growth inhibition
B) *In vivo* anticancer activity of HKBA against Ehrlich tumor					
Control	NS	10/0	21.21 ± 0.35	1266.05 ± 81.22	–
HKBA	50	7/0	20.21 ± 0.40	814.66 ± 49.30	35.65 ± 0.32
	60	7/0	20.02 ± 0.61	713.45 ± 35.82[a]	50.64 ± 0.42[b]
5 FU	22	7/0	18.85 ± 0.50	722.14 ± 50.1[a]	48.11 ± 0.31[a]
C) *In vivo* anticancer activity of HKBA against Sarcoma-180					
Control	NS	10/0	21.33 ± 0.45	1136.05 ± 73.31	–
HKBA	50	7/0	21.25 ± 0.50	723.30 ± 52.91	38.35 ± 0.62
	60	7/0	19.28 ± 0.63	659.76 ± 50.01[a]	54.36 ± 0.61[b]
5 FU	22	7/0	18.72 ± 0.49	711.22 ± 52.11[a]	46.75 ± 1.12[a]

The values reported herein are the mean values of three experiments each carried in triplicate.
a ($p < 0.05$), b ($p < 0.01$). Data are mean ± S.D.

Nuclear morphologic features

HKBA influences the nuclear morphology and leads to the formation of apoptotic bodies in HL-60 cells. Cells (2×10^5/2 ml/6 well plate) were treated with 1 and 5 µg/ml of HKBA for 18 h, stained with DAPI and visualized for nuclear morphology and apoptotic bodies using fluorescence microscope. Nuclei of untreated cells appeared round in shape (Fig.3A), while treatment with HKBA resulted in nuclear condensation and formation of apoptotic bodies (Fig.3C, D). Camptothecin (_Fig.3B), taken as a positive control also indicated condensation both in cytoplasm and nuclei. The morphological changes were accompanied by increase in number of scattered apoptotic bodies. Flow cytometric estimation of HKBA induced apoptosis and necrosis

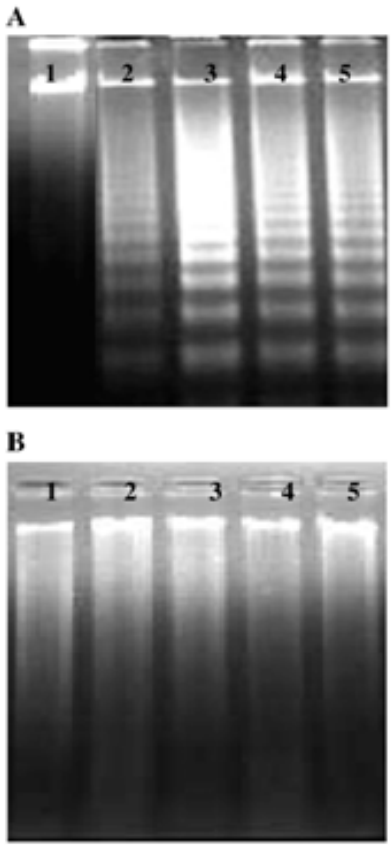


Fig. 2.

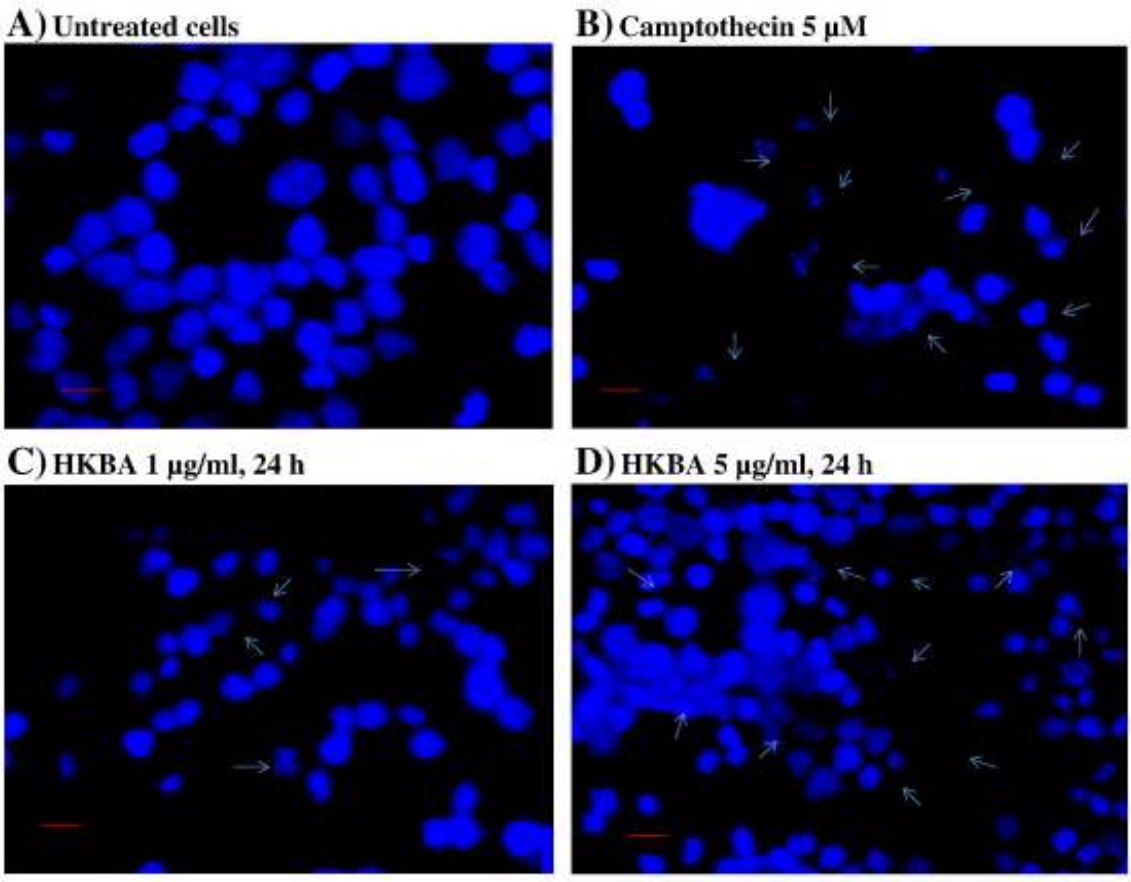


Fig. 3.

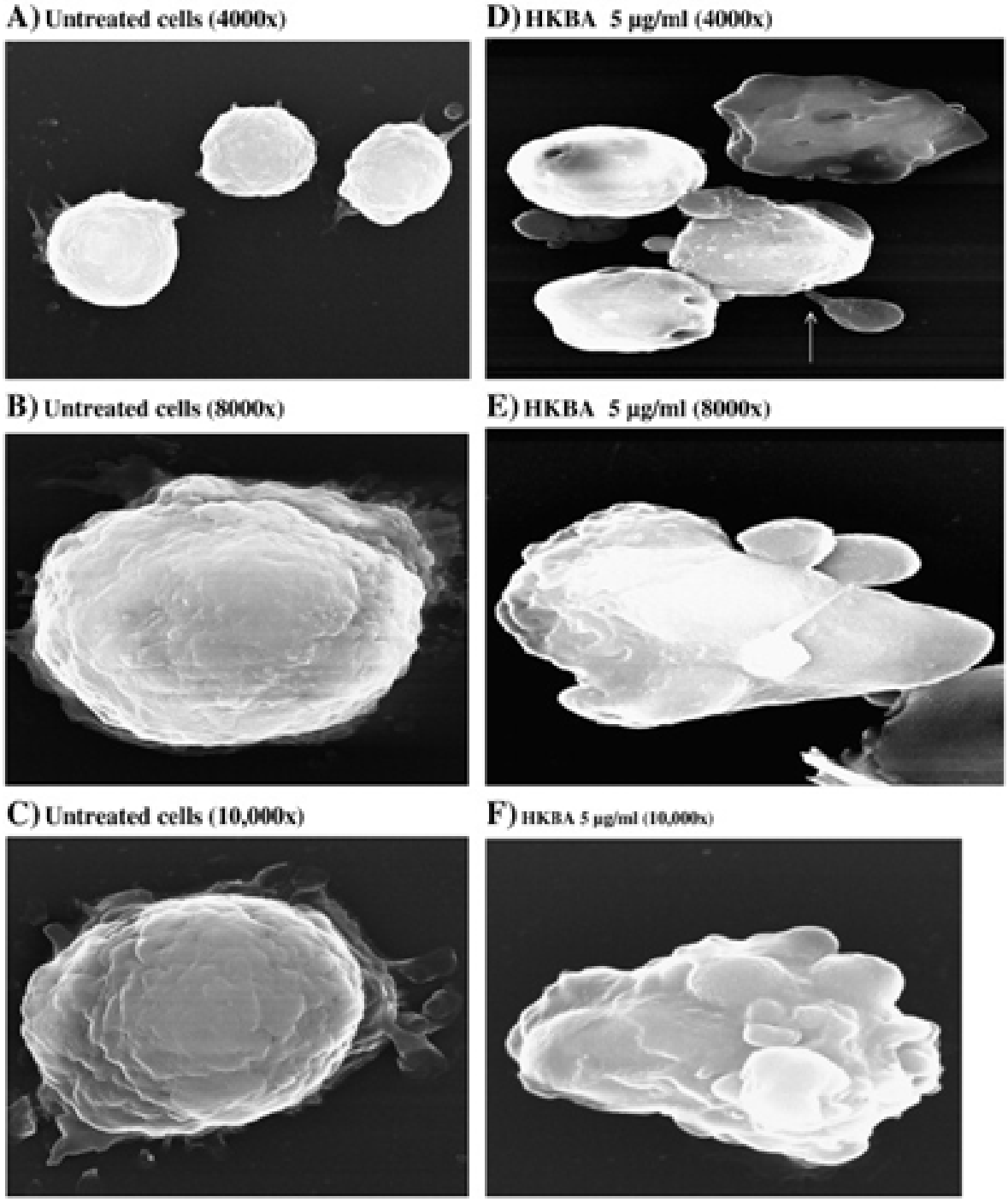


Fig. 4.

The Annexin V-FITC (stain phosphatidylserine residues)/propidium iodide (stain DNA) dual staining assay was used to detect apoptotic cells. Positive staining with Annexin V-FITC correlates with loss of membrane polarity, and the complete loss of membrane integrity leads to apoptosis or necrosis. In contrast, propidium iodide can only enter cells after loss of membrane integrity. Thus dual staining with Annexin V and PI allows clear discrimination between unaffected cells, early apoptotic cells and late apoptotic cells. After HKBA treatment, the cells were analyzed by flowcytometer. The results showed that, the basal apoptotic population in the untreated culture was 3.5%. After treatment with 5 and10 µg/ml of HKBA for 24 h, the apoptotic cell population was found to be to about 80.78% and 73.44% respectively, the necrotic population at the respective concentrations was found to be 19.22% and 26.56%. Camptothecin, used as a positive control, produced about 93.78% apoptotic population during the same exposure period (_Fig.5).

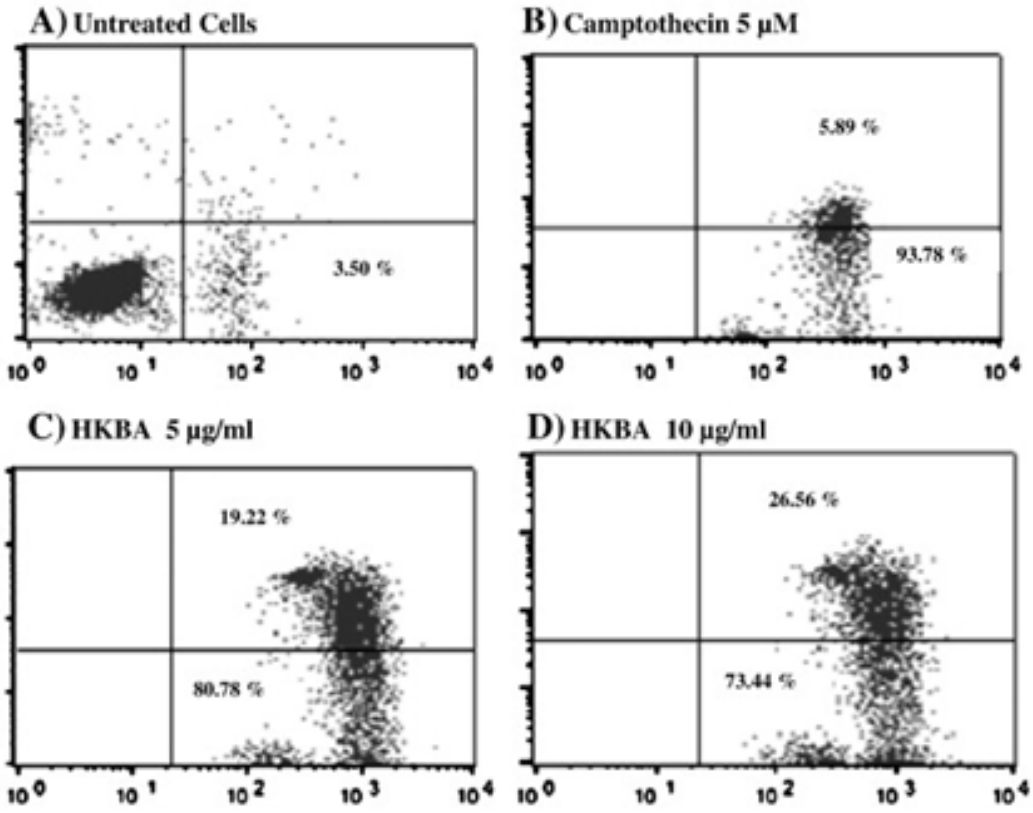


Fig. 5.

Effect of HKBA on induction of DNA double-strand breaks in HL-60 cells

Comet assay under neutral electrophoresis conditions was performed to examine nuclear DNA integrities, so that DNA double-strand breaks could be detected. DNA strand breakages in HL-60 cells were analyzed after exposure to 5 and10 µg/ml of HKBA for 24 h. The image (_Fig.6) of representative nuclei after electrophoresis of HKBA (10 µg/ml) treated cells showed formation of typical comets, with Head DNA of 68.22% and 23.42% at 5 and 10 µg/ml respectively. Tail DNA which reveals the actual DNA damage was found to be 51.78% and 66.58% at the respective concentrations. The Olive Tail movement was 6.06% and 9.56% and Tail length 18.33% and 36.21% respectively (_Table2). According to the literature ([Shao et al., 1998] and [Yasuhara et al., 2003]), a cell containing more than 50% of the total DNA in the comet tail is defined as an apoptotic cell.

Comet assay analysis of HKBA treated HL-60 cells

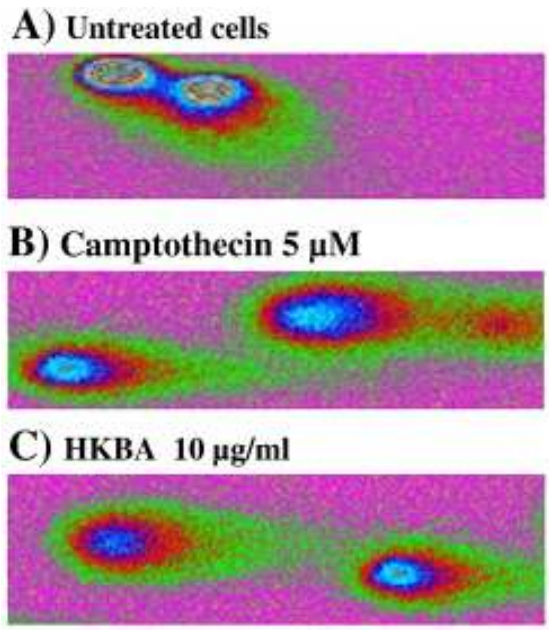


Fig. 6.

Table 2. The single cell gel electrophoresis of HKBA.

	Head DNA	Tail DNA	Olive Tail movement	Tail length
Untreated Cells	90.91 ± 0.011	9.09 ± 0.031	0.501 ± 0.04	1.34 ± 0.011
Camptothecin 5 µM	60.06 ± 0.02	39.94 ± 0.015	8.84 ± 0.022	35.13 ± 0.042
HKBA 5 µg/ml	68.22 ± 0.02	51.78 ± 0.031	6.06 ± 0.011	18.33 ± 0.021
HKBA 10 µg/ml	23.42 ± 0.04	66.58 ± 0.033	12.33 ± 0.042	36.21 ± 0.036

after 24 h of incubation revealed a head DNA of 68.22% and 23.42%, Tail DNA of 51.78% and 66.58% at 5 and 10 µg/ml respectively which signifies the DNA damage. The Olive Tail movement was 6.06% and 12.33% while a Tail length of 18.33% and 36.21% was observed at the respective concentrations. Camptothecin taken as a positive control revealed a Head DNA of 60.06% and a tail DNA of 39.94%. Data are mean S.D. from three similar experiments

Effect of HKBA on cell cycle distribution in HL-60 cells

An effective strategy to inhibit tumor growth is deregulated cell cycle progression in cancer cells. Effects of HKBA on cell cycle progression in HL-60 cells were then examined. Cells were treated with test compound at a dosage of 1, 5, and 10 µg/ml for 24 h and Fluorescence Activated Cell Sorting (FACS) analysis was followed. The DNA histogram showed that HKBA induced dose dependent increase in hypo diploid sub-G1 DNA fraction (< 2n DNA) (_Fig.7). The sub-G1 DNA fraction was 3.76% in untreated cells however after treatment with test compound (10 µg/ml) it increased to about 88.13% (_Table3). Further, HKBA was found to arrest the G_2/M phase of cell cycle.

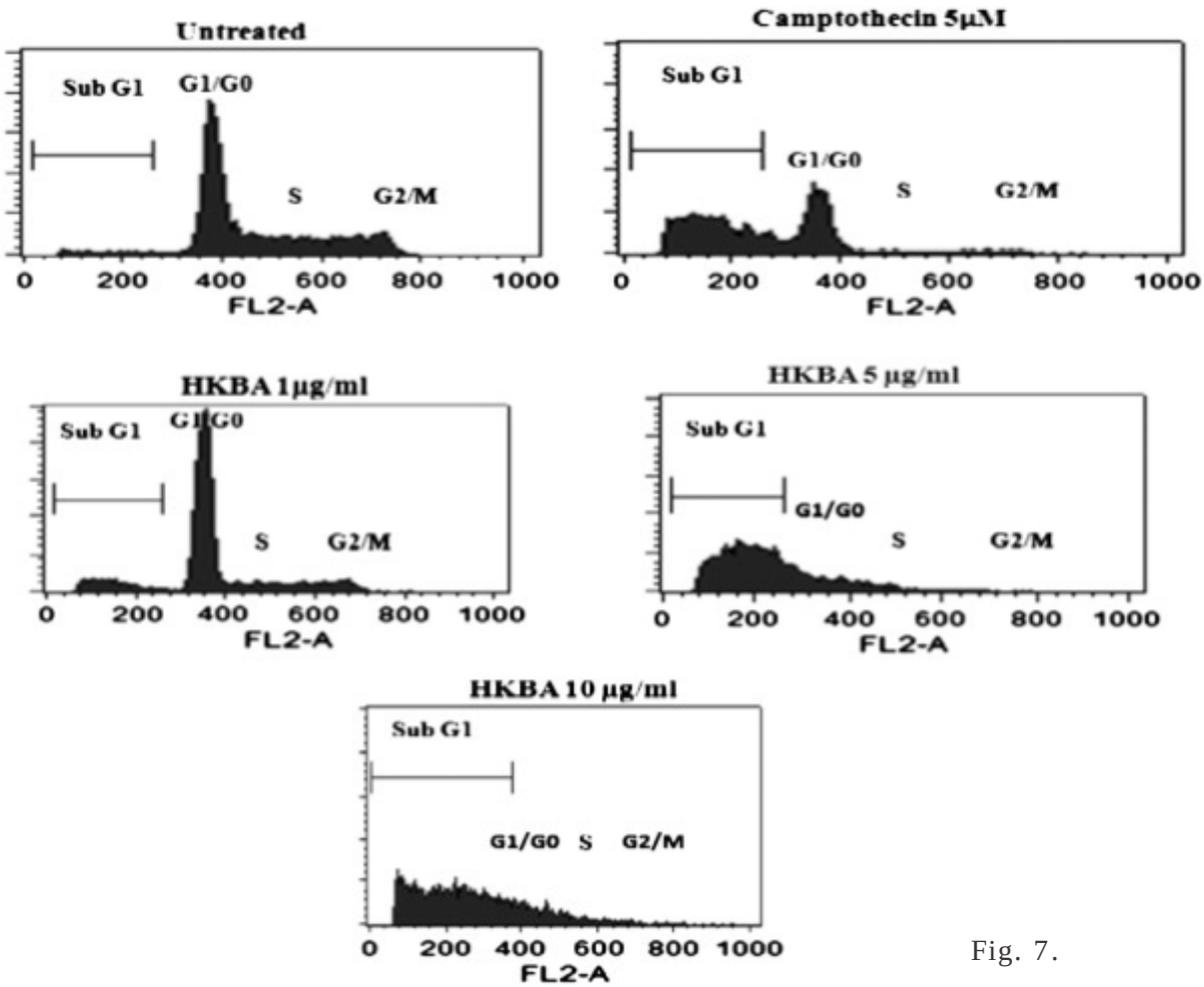


Fig. 7.

Table 3. The cell cycle phase distribution of HKBA in HL-60 cells.

Sample	Sub G_1%/(apoptotic)	G_1/G_0	S	G_2/M
Untreated cells	3.76%	47.42	23.44	28.61
Camptothecin 5 µM	56.08	32.17	6.93	5.76
HKBA 1 µg/ml	3.0%	51.42	23.34	29.21
HKBA 5 µg/ml	74.33%	12.68	17.21	4.43
HKBA 10 µg/ml	88.13%	9.63	2.11	0.89

Loss of mitochondrial membrane potential by HKBA

After rhodamine-123 incubation the fluorescent intensity, an indicator for mitochondrial membrane potential was reduced from 93.60% to 0.26% in HKBA (10 µg/ml) treated cells for 24 h, consistent with the results that HL-60 cell apoptosis occurred 24 h after treatment with HKBA at the same concentration (Fig.8).

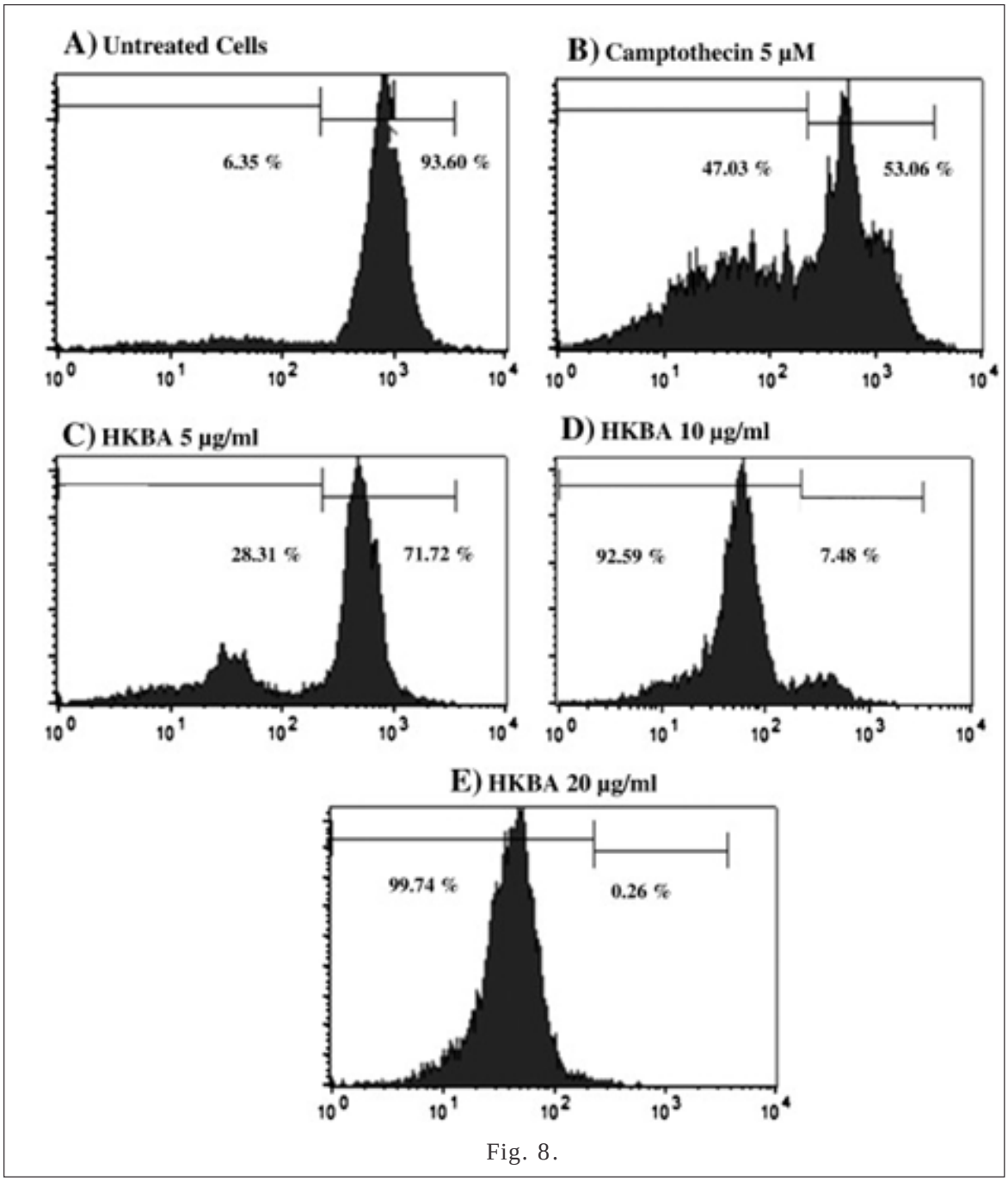


Fig. 8.

HKBA stimulates caspase activities in HL-60 cells

HL-60 cells were exposed to HKBA and evaluated for caspase-3 and -9 activities. The test compound predominately induced a dose and time dependant increase in caspase-3 and -9 activities in HL-60 cells, registering a ten-fold increase at the highest concentration of HKBA used (_Fig.9). In the light of HKBA induction of apoptosis these findings amply demonstrate the role of caspase-9 activation in up regulating downstream events leading to caspase-3 activation ([Craen et al., 1999] and [Slee et al., 1999]).

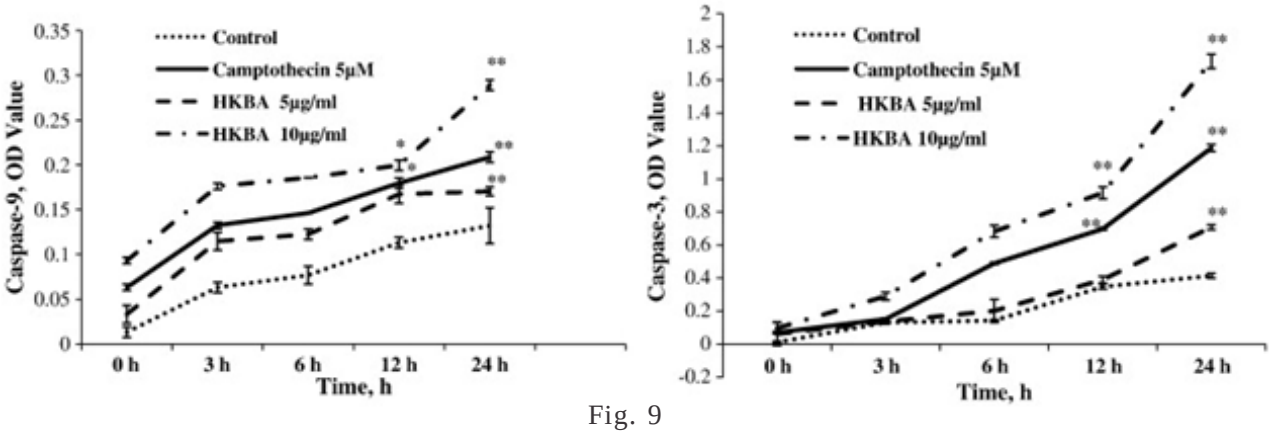


Fig. 9

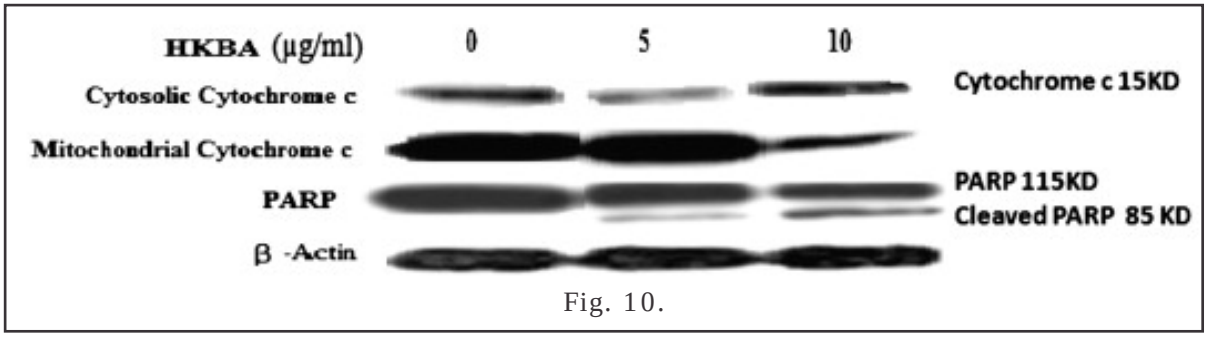


Fig. 10.

HKBA induces release of cytochrome c from mitochondria and Cleavage of PARP in HL-60 cells

HKBA induced loss of mitochondrial membrane potential in HL-60 cells was found to induce the release of cytochrome c from mitochondrion into the cytosol. The increased expression of cytochrome c in the cytosol was observed in a concentration dependant manner (Fig.10). In the further consequence of events the released cytochrome c activated caspase-9 which in turn activates caspase-3. Caspase-3 as a matter of fact uses another DNA repair enzyme, poly (ADP ribose) polymerase (PARP) as a substrate and inhibits the repair of damaged DNA. Therefore a cleaved product of PARP was observed in our study (_Fig.10). These studies thus confirm that the induction of apoptosis by HKBA was due to the activation of intrinsic pathway.

Topoisomerase I and II inhibitory assay

Topoisomerases (I and II) are the essential enzymes for proliferation of eukaryotic cells ([Nakagawa et al., 2006] and [Osheroff et al., 1983]). Drugs that target topoisomerases are among the most effective anticancer drugs. In our previous studies we found that PKBA inhibited the enzymatic activity of both the topoisomerases I and II at 20 µg/ml. The present study showed that HKBA was also able to inhibit the enzymatic activity of topoisomerases I and II, at lower concentration of 10 µg/ml (Fig.11). The inhibitory activity of HKBA was compared with the reference molecules camptothecin and etoposide for topoisomerase I and II respectively.

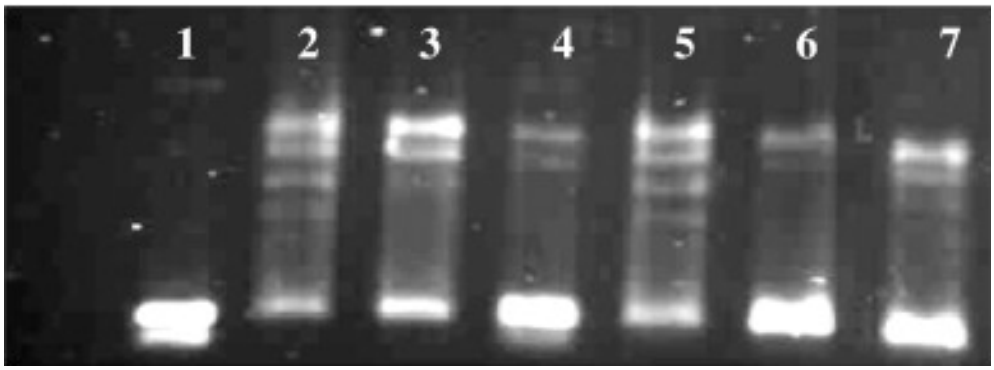


Fig. 11.

Discussion

Our previous data had shown that propionyloxy-11-keto-*β*-boswellic acid (PKBA) induced apoptosis in HL-60 cells at lower concentrations than the naturally existing acetyl-11-keto-*β*-boswellic acid (AKBA) (Chashoo et al., 2011). Since the earlier data revealed the importance of an acyl group at the 3-α-hydroxy position on the anticancer potential of KBA, it was therefore envisaged that novel analogs of KBA with increasing acyl chain length at 3-α-hydroxy position will be synthesized and subjected to anticancer studies. In this communication, butyryloxy (BKBA) and hexanoyloxy (HKBA) derivatives of KBA were synthesized. The antiproliferative potential of these analogs was evaluated in human leukemia HL-60 and MOLT-4 cell lines and it was observed that HKBA showed lower IC_{50} values than BKBA and previously reported PKBA as well. These observations prompted us to carry out detailed anticancer studies to evaluate the *in vivo* antitumor and pro-apoptotic potential of this newly synthesized analog. The *in vivo* anticancer studies of HKBA were performed in ascitic (EAC) and solid (EAT and S-180) murine tumor models. The results obtained revealed, a low dose anti-tumoral activity of HKBA than the previously synthesized PKBA (Chashoo et al., 2011) against all the three tumor models used in the study, signifying its pre-clinical importance. The pro-apoptotic effect of HKBA was demonstrated by various hallmarks including DNA fragmentation, apoptotic body's formation and Annexin-V binding in human leukemia HL-60 cells. It was also observed that HKBA treatment arrested cancer cells at the G_2/M phase and showed significant increment in the Sub G_1 phase of the cell cycle. The literature precedent and the kinetic data available have indicated that mitochondrion undergoes major changes in membrane integrity before classical signs of apoptosis become manifest (Gruber et al., 2004). It is

widely accepted that the alterations of mitochondrial function, such as breakdown of the mitochondrial transmembrane potential (Ψm) and release of proapoptotic cytochrome c, constitute key events of the mitochondrial apoptosis signaling cascade (Susin et al., 1998). In the current study, HKBA was found to induce changes in the mitochondrial transmembrane potential which was found to be associated with the release of cytochrome c from mitochondria into the cytosol. The released cytochrome c from the mitochondrion resulted in the upregulation of the initiator caspase-9 which in turn activated the executioner caspase-3. Once activated, caspase-3 induced cleavage of poly (ADP-ribose) polymerase (PARP) thus prevented any unwanted repair of the damaged DNA. Moreover, the cleavage of PARP is consistent with DNA fragmentation, which results in the morphologic and biochemical features of apoptosis. These findings therefore indicate that mitochondrial signaling pathway participate in the HKBA induced apoptosis. Although, boswellic acids have repeatedly been reported to induce apoptosis in cancer cells, however, the results obtained from the current study demonstrated the low dose apoptotic potential of a newly synthesized analog, HKBA. Since our previous data showed that, PKBA induced cancer cells to death by inhibiting the enzymatic activity of topoisomerases I and II, HKBA was therefore subjected to topoisomerase inhibitory studies, where it was found to inhibit the catalytic activity of both topoisomerases I and II at low doses. Several reports have revealed that chemotherapeutic agents that target topoisomerases I and II set in motion a series of biochemical changes that culminate in cell death (Bielawski et al., 2006). These antitumor drugs interact with both topoisomerases I and II simultaneously. This mechanism of action appears to be advantageous, because selective inhibition of topoisomerase I has been reported to increase topoisomerase II enzyme activity and *vice-versa*, which may be important for the development of drug resistance. In this regard, a single compound able to inhibit both topoisomerase I and II may present the advantage of improving anti topoisomerase activity, with reduced toxic side effects, with respect to the combination of two inhibitors (Salerno et al., 2010). Although, dual inhibitors theoretically overcome these problems, however, some of the inhibitors like Intoplicin and XR11576 have been found to be associated with liver toxicity, diarrhea, nausea, vomiting and alopecia ([De Jonge et al., 2004] and [Salerno et al., 2010]). Besides this, the chemopreventive effects of well known topoisomerase targeting drugs such as camptothecin and etoposide have also been found to be associated with multiple and severe side effects such as myelosuppression, thrombocytopenia, anemia, bone marrow and gastrointestinal toxicity (Hartman and Lipp, 2006). Boswellic acids in contrast to this are known to have good safety profile showing no severe side effects ([Gupta et al., 1997], [Mantle et al., 2001] and [Safayhi et al., 2000]). In addition, we reported recently that PKBA was non-toxic to the normal cells, the present investigation also showed that in spite of the increased anticancer potency at lower doses, HKBA seems to be selective for tumor cells, since no apoptosis was observed in normal cells. In summary, this paper constitutes the first instance for the demonstration of anticancer activity of a newly synthesized 3-α-hexanoyloxy derivative of KBA, HKBA. The above data revealed that, compared to the previously reported boswellic acids, HKBA showed a low dose antiproliferative and pro-apoptotic potential in human leukemia HL-60 cells. Besides this, a low dose antitumor potential in ascitic and solid murine tumor models was also observed. Keeping all this into consideration, whether the anticancer effects HKBA at low doses characterized here have any clinical relevance, remains to be determined. Further, pharmacokinetic studies should address this problem.

Bakuchiolderivativesas novelandpotentcytotoxicagents:Areport

Rabiya Majeed, Mallepally V.Reddy, Praveen K. Chinthakindi, PayareL. Sangwan, AbidHamid*, Gousia Chashoo, AjitK. Saxena, Surrinder Koul

Natural products/molecules derived from plants have beenareliablesourceoftherapeuticagentsfortheuse inhumansandthequesttoswellthesenumbersgoes unabated. Apartfromtheuse of plantderivedmoleculesdirectlyas drugssuchasvincristine, vinblastine, reserpine, etoposide, artemisinin; manyplantderivedscaffolds, throughtailoringby thechemists, have resultedin the developmentof someof themosteffectivedrugse.g. khellinto sodium chromoglycateananti-asthmatic drug, papaverineto verapamilforhypertension, galegine tometforminfor diabetes. Therefore, theexploration of plants for theirpossiblemedicinaluse needsto be continued. Recently, we carried out a studyon bakuchiolthemajorchemicalconstituent of P. corylifolia. Bakuchiolisreportedascytotoxicto ward breastcancer with IC50 8.29x103mol/L (MDA-MB-231)and 2.89x105mol/L (T-47D). However, adetailedinvestigationofthismoleculeanditsderivatives is still awaited. All the synthesized derivatives weretestedat 50

µMconcentrationfortheirabilitytoinduce cytotoxicityinhumancancercellline sencompassinglung (A-549), breast (MCF-7), prostate(PC-3), cervical(HeLa), leukemia (THP-1), CNS/neuroblastoma (IMR-32), and ovarian (OVCAR-5) cell lines, takingmitomycin/ adriamycin/ 5-FUasthegoldstandard.

Themoleculesthatexhibited > 50%inhibition werescreenedat lowerconcentration(30 µM)whichresultedintheidenti?cation of 17, 22, and2 as themostpotentmolecules(Table 2)andthese werefurthertestedfortheirinhibitory potentialat lowerconcentrations(20, 10 and5 µM).Whilecompound 2showed57%growthinhibitionat 20 µMagainstcoloncancercell line, 17and22 showed inhibitionat 20 µMagainstallthecancercelllines.Compound17displayedinhibitionagainstfourcancercelllinesand22against threecelllinesat10 µM.Boththecompoundsat5 µMconcentration,showedinhibitionly againstTHP-1cellline (Table 3). Since leukemiccelllineswerefoundthe mostsensitivecellstowardthecytotoxicpotentialof thesecompounds,theIC_{50} valuewasfurthercalculatedattwotimepoints(48hand24h)onHL-60cells using MTT assay. Both compounds 17 and 22 showed concentrationand time dependent inhibition of cell proliferation displaying theIC50 values 1.8 µM and 18 µM for 17 while 2.0 µM and 16 µM for 22after 48 h and 24 h time incubation respectively (Figs. 2 and 3).Further experiments were carried out to verify whether thecancer cell death induced by the 17 and 22 was apoptotic, as itbecame increasingly evident that although the primary intracellulartargets and the pharmacological mechanisms of action ofthe anti-cancer drugs vary vastly, the drug induced cell killing is generally mediated by apoptosis. Compounds 17 and 22 were observed to be potent apoptosis inducers, as evidenced from the measurement of two important biological end-points of the apoptosis viz., DNA fragmentation and increase in sub-G0 DNA fraction. The apoptotic potential of 17 and 22 was confirmed through induction of DNA fragmentation in HL-60 cells, which is known as the hallmark of apoptosis. Compound 17 induced the laddering pattern of apoptosis at a concentration of 20 µM, and in 22, the laddering pattern was concentration dependent. The minimal concentration inducing DNA fragmentation of 20 µM, which on extension to 40 µM showed no smear formation which is a representative of post apoptotic necrosis (Figs. 4 and 5).

Further extending our study, DNA cell cycle analysis was performed using HL-60 cells. Most of the differentiated cells are arrested in the G1 phase but in case of cancer cells, this control is lost and they go on dividing. Thus, 17 and 22 were subjected to hypo-diploid sub-G0 DNA fraction (< 2nDNA) analysis as a measure of apoptosis. HL-60 cells treated with 17 and 22 at 30 µM concentration showed a considerable increase in the hypodiploid sub-G0 DNA fraction (< 2nDNA), i.e. 68% in case of 17 and 66% in case of 22 at 24 h treatment, indicating DNA damage (Figs. 6 and 7). To rule out whether the compounds have any effect on the mitochondrial functioning, mitochondrial membrane depolarization assay was performed, both 17 and 22 caused the disruption of mitochondrial membrane and subsequent loss of

Table 2
Cytotoxic activity[a] (%age growth inhibition) of bakuchiol and its selected derivatives at two different concentration against various human cancer cell lines.

Compounds	Conc. (µM)	Breast MCF-7	Liver Hep-2	Lung A-549	Prostate PC-3	Ovary OVCAR-5	CNS IMR-32	Cervical HeLa	Leukemia THP-1
1	50	**64 ± 1**	**53 ± 1**	**65 ± 2**	**59 ± 1**	**67 ± 1**	**61 ± 3**	**56 ± 1**	**76 ± 2**
	30	33 ± 1	20 ± 1	30 ± 2	21 ± 3	25 ± 3	31 ± 4	20 ± 1	44 ± 1
2	50	**77 ± 2**	**52 ± 3**	**72 ± 3**	**69 ± 2**	**71 ± 2**	**71 ± 3**	**65 ± 1**	**81 ± 1**
	30	**56 ± 3**	**51 ± 2**	**60 ± 2**	**60 ± 3**	**62 ± 2**	**59 ± 3**	45 ± 1	**63 ± 1**
8	50	26 ± 2	30 ± 1	**60 ± 1**	**60 ± 2**	**62 ± 2**	**53 ± 1**	**53 ± 2**	**64 ± 2**
	30	12 ± 1	16 ± 1	33 ± 1	27 ± 1	29 ± 2	22 ± 2	24 ± 2	39 ± 2
10	50	**51 ± 2**	40 ± 1	**53 ± 1**	**55 ± 3**	**57 ± 2**	**53 ± 2**	**50 ± 1**	**58 ± 3**
	30	37 ± 3	22 ± 1	34 ± 2	31 ± 1	29 ± 2	23 ± 2	28 ± 1	29 ± 2
17	50	**79 ± 2**	**65 ± 1**	**78 ± 2**	**88 ± 2**	**76 ± 1**	**85 ± 3**	**69 ± 1**	**95 ± 2**
	30	**58 ± 2**	**53 ± 1**	**61 ± 2**	**62 ± 2**	**55 ± 1**	**57 ± 2**	**52 ± 1**	**68 ± 1**
22	50	**77 ± 2**	**62 ± 1**	**72 ± 2**	**94 ± 1**	**67 ± 1**	**71 ± 2**	**80 ± 2**	**86 ± 2**
	30	**52 ± 2**	45 ± 1	**55 ± 3**	**50 ± 2**	**50 ± 1**	**51 ± 2**	**56 ± 2**	**60 ± 1**
24	50	48 ± 2	46 ± 1	**60 ± 3**	**61 ± 2**	18 ± 2	30 ± 2	27 ± 2	**51 ± 2**
	30	28 ± 3	32 ± 1	33 ± 2	15 ± 1	8 ± 2	14 ± 2	5 ± 2	32 ± 1
5-FU[b]	20	–	–	**76 ± 2**	**76 ± 1**	**78 ± 3**	**81 ± 2**	**74 ± 3**	**91 ± 3**
Mitomycin[b]	1	–	**81 ± 3**	–	–	–	–	–	–
Adriamycin[b]	1	**85 ± 3**	–	–	–	–	–	–	–

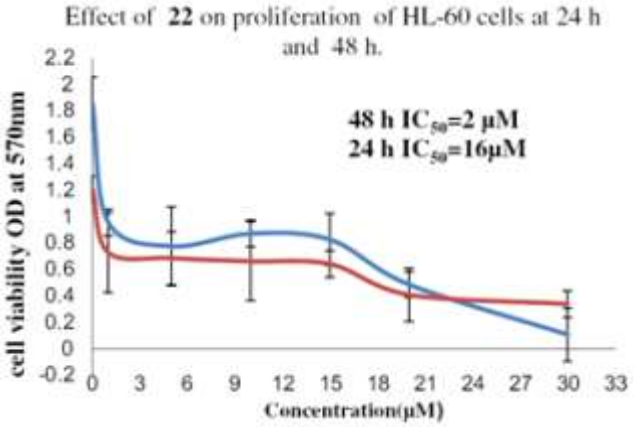
The bold values are shown for those compounds which have proved to be active and those in normal font represent least significant.
[a] Results are mean ± SD of three separate experiments, conducted in triplicate at the concentration of 50 µM.
[b] Concentration of 5-FU = 20 µM, Mitomycin = 1 µM, Adriamycin = 1 µM.

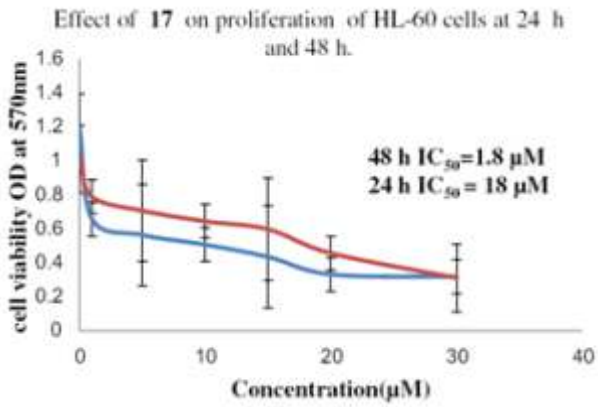
Table 3
Cytotoxic activity[a] (%age growth inhibition) of bakuchiol derivatives at 20 μM, 10 μM and 5 μM concentration against human cancer cell lines.

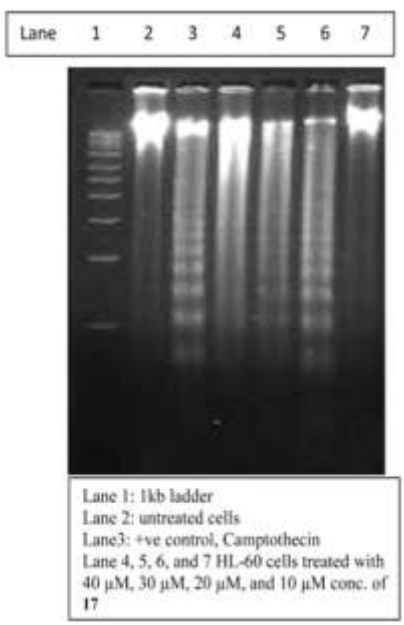
Compounds	Conc. (μM)	Breast MCF-7	Liver HEP-2	Lung A-549	Prostate DU-145	Leukemia THP-1	Prostrate PC-3	CNS IMR-32	Colon HCT-15
2	20	44 ± 2	46 ± 2	1 ± 2	42 ± 2	50 ± 2	45 ± 3	48 ± 3	**57 ± 2**
	10	31 ± 2	30 ± 1	22 ± 1	29 ± 2	44 ± 2	32 ± 1	34 ± 2	46 ± 1
	5	15 ± 2	14 ± 3	13 ± 2	19 ± 1	34 ± 1	21 ± 2	27 ± 2	31 ± 2
17	20	**50 ± 2**	**50 ± 1**	**53 ± 3**	**54 ± 2**	**76 ± 2**	**57 ± 2**	**62 ± 2**	**63 ± 3**
	10	32 ± 1	34 ± 3	40 ± 2	**50 ± 1**	**65 ± 3**	35 ± 2	**50 ± 1**	**55 ± 3**
	5	27 ± 2	17 ± 2	32 ± 3	32 ± 3	**54 ± 2**	24 ± 4	46 ± 1	29 ± 3
22	20	**50 ± 3**	**58 ± 4**	**69 ± 1**	**73 ± 2**	**78 ± 4**	**58 ± 2**	40 ± 3	**61 ± 3**
	10	37 ± 1	46 ± 1	47 ± 1	**57 ± 1**	**62 ± 1**	41 ± 1	32 ± 1	**50 ± 2**
	5	24 ± 1	23 ± 1	24 ± 1	20 ± 4	**53 ± 2**	23 ± 2	13 ± 1	28 ± 3
5-Fu[b]	20	–	–	**78 ± 2**	**75 ± 1**	**85 ± 3**	**75 ± 3**	**83 ± 2**	**63 ± 1**
Mitomycin[b]	1	–	**78 ± 3**	–	–	–	–	–	–
Adriyamycin[b]	1	**91 ± 1**	–	–	–	–	–	–	–

The bold values are shown for those compounds which have proved to be active and those in normal font represent least significant.
[a] Results are mean ± SD of three separate experiments, conducted in triplicate at the concentration of 50 μM.
[b] Concentration of 5-FU = 20 μM, Mitomycin = 1 μM, Adriamycin = 1 μM.

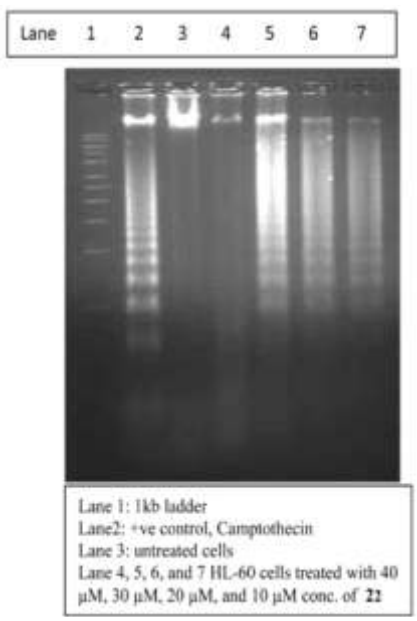
mitochondrial membrane potential in a concentration dependent manner. Compound 17 showed almost 54% of dissipation of mitochondrial membrane potential and 22 showed 55.4%, the untreated control showed only 6% and cells treated with camptothecin showed 55.5% (at 5 μM) suggesting the central role of mitochondria toward the apoptotic potential of both of these molecules (Figs. 8 and 9).



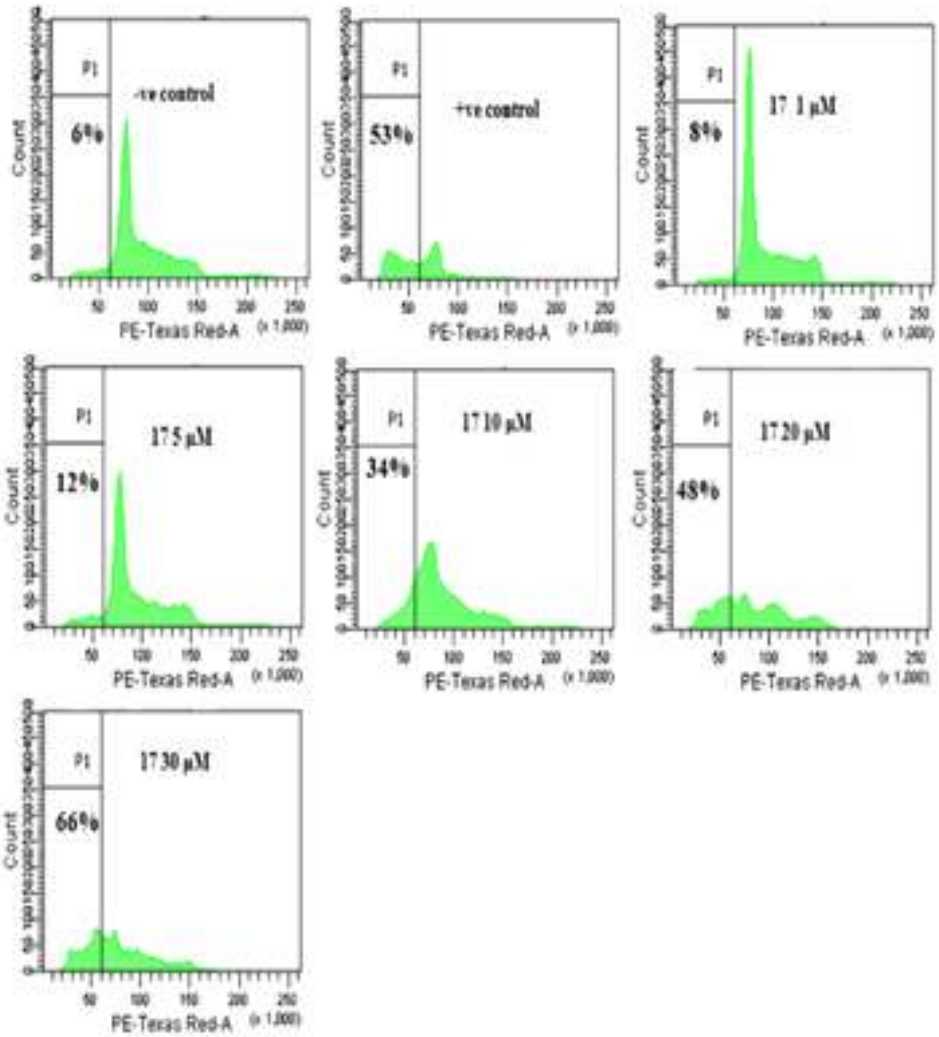


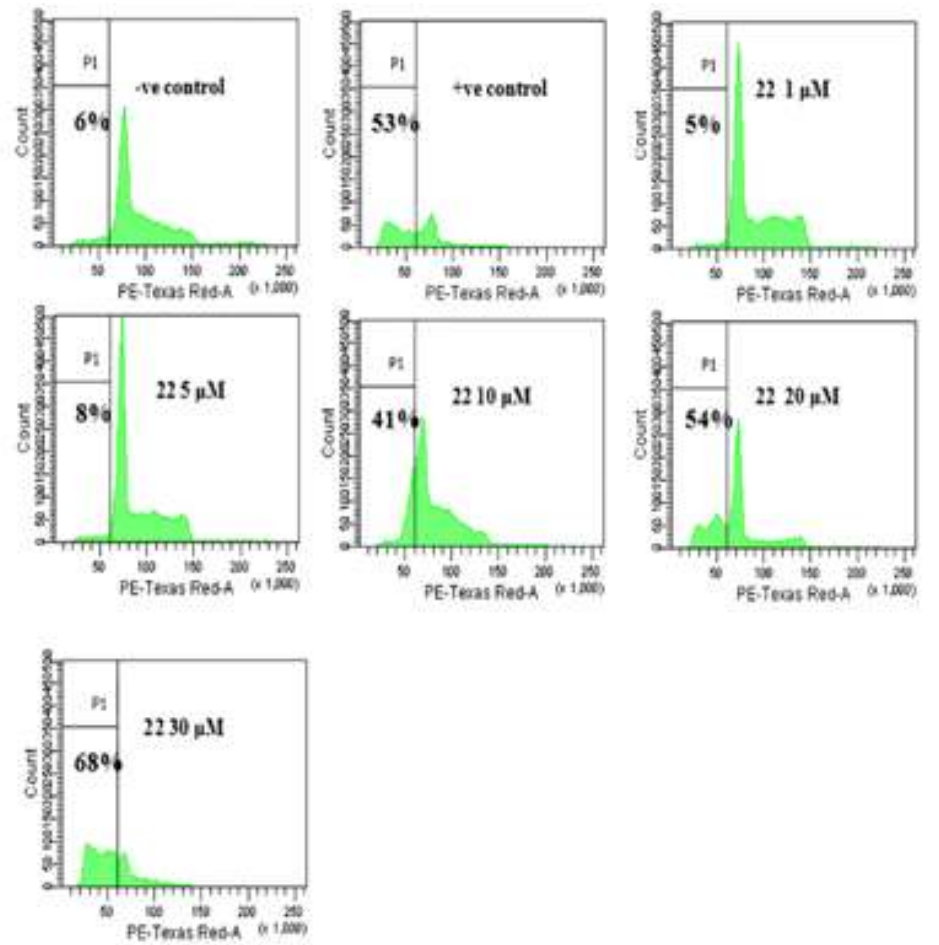


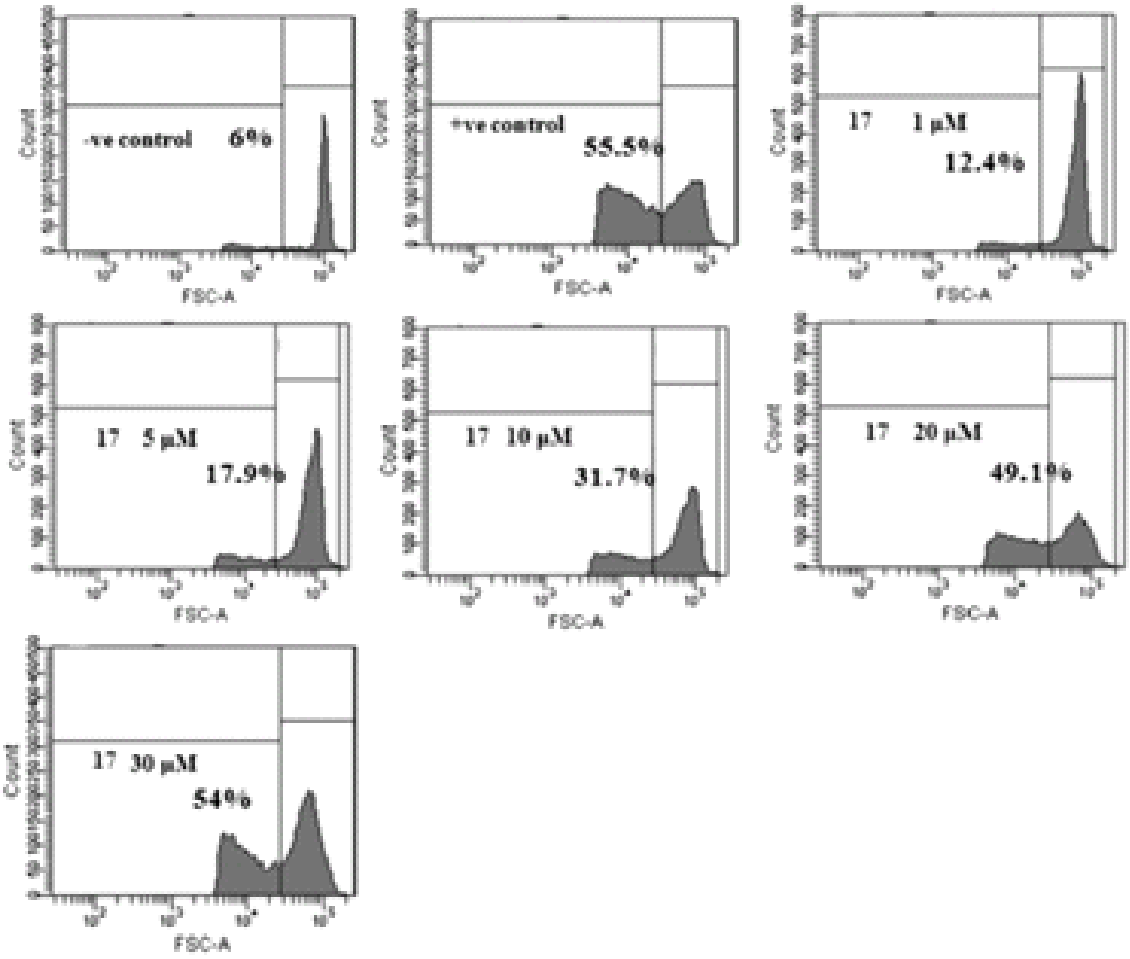
Lane 1: 1kb ladder
Lane 2: untreated cells
Lane3: +ve control, Camptothecin
Lane 4, 5, 6, and 7 HL-60 cells treated with 40 μM, 30 μM, 20 μM, and 10 μM conc. of **17**

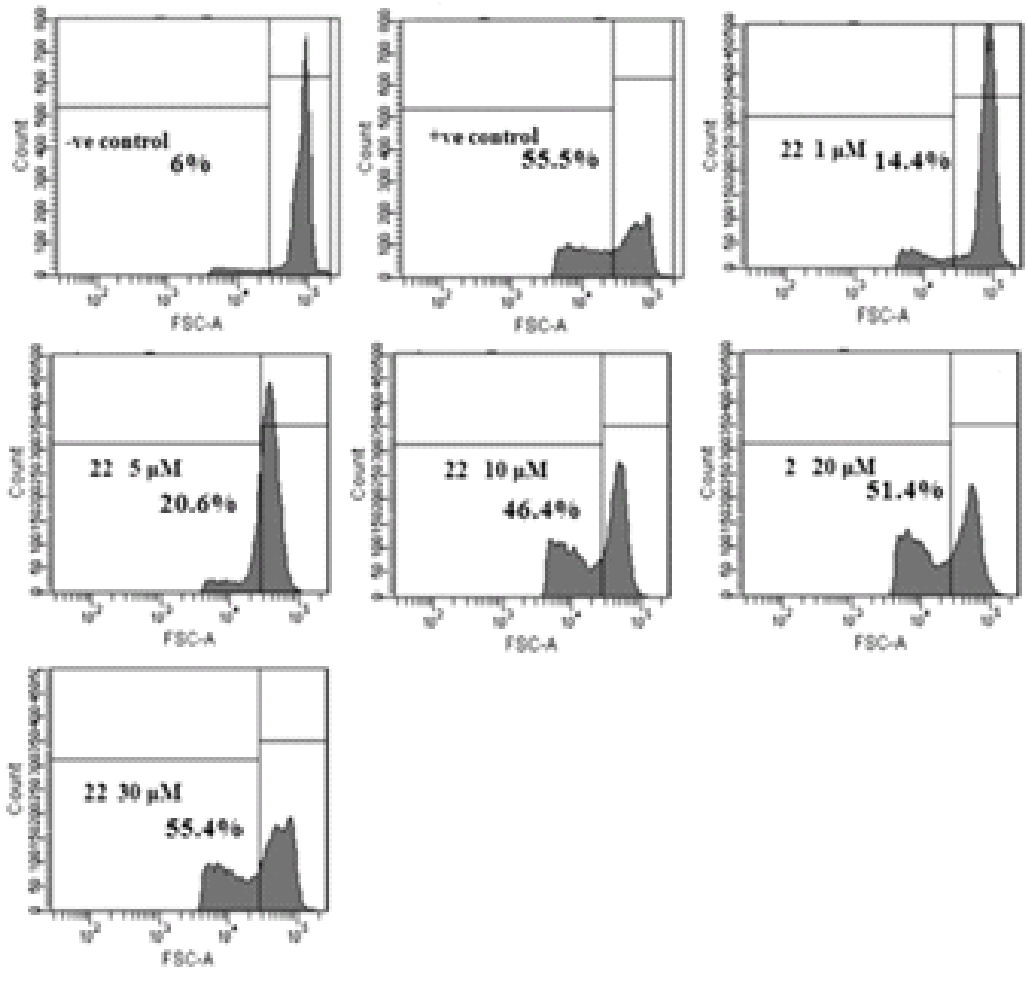


Lane 1: 1kb ladder
Lane2: +ve control, Camptothecin
Lane 3: untreated cells
Lane 4, 5, 6, and 7 HL-60 cells treated with 40 μM, 30 μM, 20 μM, and 10 μM conc. of **22**


P1
-ve control
6%
+ve control
53%
17 1 µM
8%
17 5 µM
12%
17 10 µM
34%
17 20 µM
48%
17 30 µM
66%
Count
PE-Texas Red-A


P1
-ve control
6%
+ve control
53%
22 1 µM
5%
22 5 µM
8%
22 10 µM
41%
22 20 µM
54%
22 30 µM
68%
Count
PE-Texas Red-A


-ve control 6%
+ve control 55.5%
17 1 µM 12.4%
17 5 µM 17.9%
17 10 µM 31.7%
17 20 µM 49.1%
17 30 µM 54%
Count
FSC-A


-ve control 6%
+ve control 55.5%
22 1 µM 14.4%
22 5 µM 20.6%
22 10 µM 46.4%
2 20 µM 51.4%
22 30 µM 55.4%
Count
FSC-A

PARPcleavageand perturbanceinmitochondrial membranepotentialby3-a-propionyloxy-b-boswellicacid resultsincancercelldeathandtumorregressioninmurinemodels

Yasrib Qurishi, AbidHamid, Parduman R Sharma, Zahoor Ahmad Wani, Dilip M Mondhe, Shashank K Singh, Mohmmad Afzal Zargar, Samar S Andotra, Bhahwal Ali Shah, Subhash C Taneja and Ajit Kumar Saxena*

Apoptoticinductionincancercellshas becomeamajorfocusof anticancertherapeutics. Inthisregard, β-boswellicacids, naturallyoccurring pentacyclictriterpenes, havedemonstratedantiproliferativea ndcytotoxiceffects againstdifferenttypesofcancers. Surprisingly, notmuch has beenreportedregardingthechemical modificationsorpreparationofstructu ralanalogsofthekeyconstituents of β-boswellic acid. Results: Thecytotoxicitydatarevealedthesensiti vity ofvarioushumancancercelllinesofvari edtissueorigintob-boswellicacid, which robustlyinducedcellcyclearrest, DNAfragmentationand lossofmitochondrial membranepotential. MorphologicalstudiesoftheeffectsofP OBArevealedloss ofsurfaceprojections,chromatinconde nsation,apoptoticbody formationand POBA-mediatedPARP cleavage.For*invivo*therapeuticexperime nts,murinetumor modelsweretreatedwithPOBAandthetre atmentresultedinasignificantlyhigher levelofgrowthinhibitionandapoptosiswa ssignificantlyinduced.Conclusion: Thesefindingsdemonstratethatacylsubst ituents/groupsinthemainskeletonof b-boswellicacidhave thepotentialtobepotentchemotherapeut icagents.

A novel parthenin analog exhibits anti-cancer activity: Activation of apoptotic signaling events through robust NO formation in human leukemia HL-60 cells

Ajay Kumar, Fayaz Malik, Shashi Bhushan, Bhahwal A. Shah, Subhash C. Taneja, Harish C. Pal, Zahoor A. Wani, Dilip M. Mondhe and Jagdeep Kaur

This study describes the anti-cancer activity of P19, an analog of parthenin. P19 induced apoptosis in HL-60 cells and inhibited cell proliferation with 48 h IC50 of 3.5 μM. At 10 mg/kg dose, it doubled the median survival time of L1210 leukemic mice and at 25 mg/kg it inhibited Ehrlich ascites tumor growth by 60%. Investigation of the mechanism of P19 induced apoptosis in HL-60 cells revealed that N-acetyl-L-cysteine (NAC) and s-methylisothiourea (sMIT) could reverse several molecular events that lead to cell death by inhibiting nitric oxide (NO) formation. It selectively produced massive NO in cells while quenching the basal ROS levels with concurrent elevation of GSH. P19 disrupted mitochondrial integrity leading to cytochrome c release and caspase-9 activation. P19 also caused caspase-8 activation by selectively elevating the expression of DR4 and DR5. All these events lead to the activation of caspase-3 leading to PARP-1 cleavage and DNA fragmentation. However, knocking down of AIF by siRNA also suppressed the apoptosis substantially thus indicating caspase independent apoptosis, too. Further, contrary to enhanced iNOS expression, its transcription factor, NF-kB (p65) was cleaved with a simultaneous increase in cytosolic IkB-alpha. In addition, P19 potently inhibited pro-survival proteins pSTAT3 and survivin. The multimodal pro-apoptotic activity of P19 raises its potential usefulness as a promising anti-cancer therapeutic.

2-Anilinonicotinyl linked 2-aminobenzothiazoles and [1,2,4] triazolo[1,5-b] [1,2,4] benzothiadiazine conjugates as potential mitochondrial apoptotic inducers

Ahmed Kamal, Y.V.V. Srikanth, M. Naseer, Ahmed Khan, Md. Ashraf, M. Kashi Reddy, Farheen Sultana, Tandeep Kaur, Gousia Chashoo, Nitasha Suri, Irum Sehar, Zahoor A. Wani, Arpita Saxena, Parduman R. Sharma, Shashi Bhushan, Dilip M. Mondhe and Ajit K. Saxena

A series of N-(2-anilino-pyridyl) linked 2-amino benzothiazoles (4a–n) and [1,2,4]triazolo [1,5-b] benzothiadiazine conjugates (5a–j) have been designed, synthesized and evaluated for their antiproliferative activity. Some of these compounds (4h–k, 4n, and 5e) have exhibited potent cytotoxicity speci?cally against human leukemia HL-60 cell lines with IC50 values in the range of 0.08–0.70 μM. All these compounds were tested for their effects on the cell cycle perturbations and induction of apoptosis. Morphological evidences of apoptosis, including fragmentation of nuclei and inter nucleosomal DNA laddering formation were clearly observed after 24 h exposure to compound 4i. Flow cytometry analysis revealed that compound 4i showed drastic cell cycle perturbations due to concentration dependant increase in the sub-G0 region which comprises of both the apoptotic and debris fraction, thus implying the extent of cell death. These compounds trigger the mitochondrial apoptotic pathway that results in the loss of mitochondrial membrane potential through activation of multiple caspases followed by activation of caspase-3, and ?nally cleavage of PARP. Further the mechanism of cell death was analysed by ?uorescent microscopic analysis and also by scanning electron microscopy. The cytotoxicity of 4i correlated with induction of apoptosis, caspases activation and DNA damage and thus indicating the apoptotic pathway of anticancer effect of these compounds.

5. INSILICO BIOLOGY

5.1 Molecular modeling studies on IGF 1R "Insulin Like Growth Factor 1Receptor" for the identification of its inhibitors.

Priya Mahajan, Amit Nargotra, Sravan Kumar, Rammohan, Parvinder Pal Singh, Sangpal Sawant, Anindya Goswami and Ram Vishwakarma.

In order to identify novel inhibitors of IGF 1R, a total of 17 3D crystal structure of IGF1R were downloaded and based on the resolution and location of co-crystalised ligand, PDB ID 2OJ9 bound with Benzimidazole Inhibitor (BMI) was selected for docking studies. The identification of structures inhibiting IGFR1 was carried out using the following two approaches:-

i. Virtual modification of a selected ligand (scaffold from Medicinal Chemistry Division) was carried out involving the docking studies on 2OJ9. Five molecules with better affinity than the selected molecule were shortlisted and have been proposed for their synthesis.

ii. Molecular docking studies of the drug like compound library (20,000 nos) was carried out on the 3D crystal structure of IGFR1 (PDB ID 2OJ9), for identification of potent hits. In total, 26 structures showing better affinity (than standard) towards IGFR1 were identified. These compounds have been submitted for wet lab bioevaluation studies.

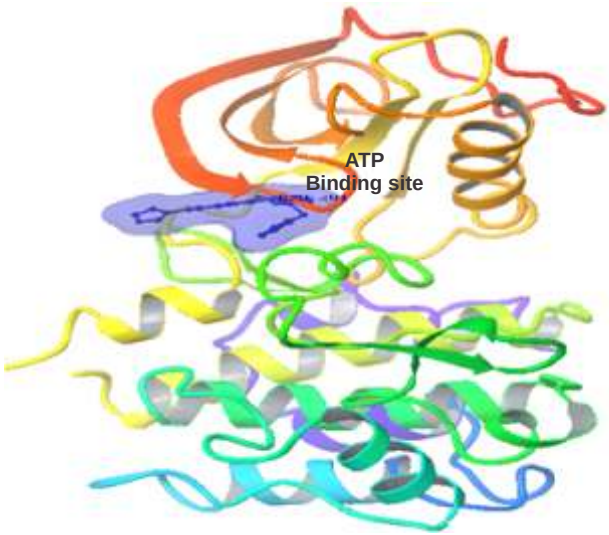


Figure 1. Structure of β subunit of IGFR1with ATP Binding Site

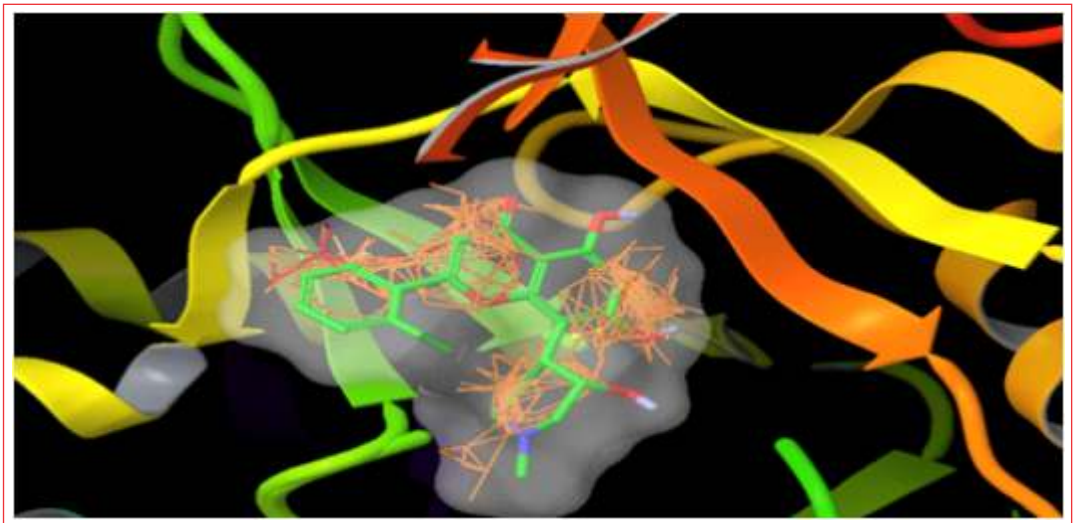


Figure 2.Hot spots or clefts (C1, C2 & C3) within the binding pocket of CDK2

5.2 Fragment based studies on designing CDK2 inhibitors

Priya Mahajan, Amit Nargotra, Rammohan, Laksma Reddy, Umed Singh, Sudhakar, Parvinder Pal Singh, Sangpal Sawant and Ram Vishwakarma.

The availability of the large number of 3D crystal structures of CDK2 co-crystalised with more than 150 different ligands makes it very attractive target from informatics point of view. Therefore, in order to identify a potent CDK2 inhibitors, fragment based studies have been carried out wherein the co-crysalised ligands (of all the available crystal structures) were splitted into several fragments and incorporated into the fragment library of Schrodinger software, thereby enriching the existing fragment library. Using FT-Map server, several different clefts (hot spots) within the binding pocket of CDK2 were identified (figure 2) and the fragement library was docked onto these clefts. The good scoring fragments were linked and several virtual compounds with better affinity towards CDK2 were identified. Further analysis to design specific CDK2 inhibitors using rational drug discovery approach is in progress.

5.3 Pharmacophore modeling of RV1258c inhibitors

Rukmankesh, Amit Nargotra, Inshad Ali Khan, Surrinder Koul. Figure 3.A general 4-point pharmacophore obtained for all the active compounds against Rv1258c

The activity data of several molecules (from IIIM repository) known to possess Rv1258c inhibitory activity through wet lab results were used for developing a pharmacophore model for Rv1258c inhibitory activity. Three different models viz., 3-point, 4-point and 5-point pharmacophore models were developed. Finally, a four point pharmacophore model (as shown in figure 3) that covered the maximum number of molecules, in the training set, was generated. This model would be useful for filtering potent inhibitors of the said target, from several compound libraries, which would be useful towards the identification of efficacy enhancers for Anti-TB drug.

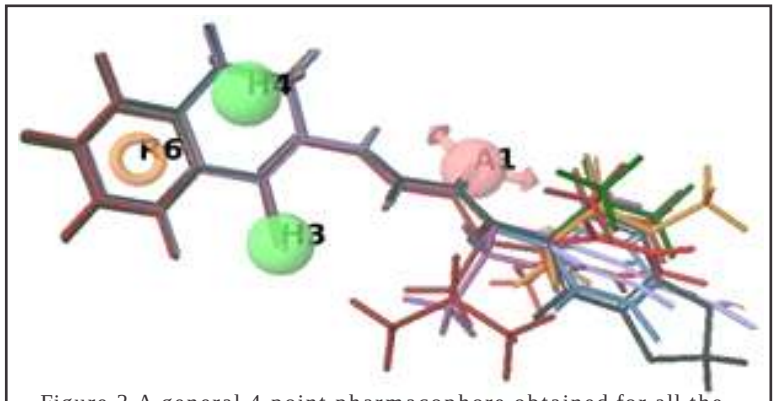


Figure 3.A general 4-point pharmacophore obtained for all the active compounds against Rv1258c

5.4 Development of Institutional repository database comprising of Natural products, Procured drug like compound library, synthetic and semi-synthetic compounds (in-house) with sub-structural search facility.

Monika Gupta, Amit Nargotra, Naresh Satti and Ram Vishwakarma.

For any drug discovery programme, a repository of compounds is very essential. In order to develop such a platform, a web-enabled database with sub-structural search facility has been developed for the existing drug like compound library (procured from Chembridge), natural products, synthetic and semi-synthetic compounds preparedwithin the Institute. This database is regularly updated. It also provides information about the latest entries as well as the number of compounds, including their structures, submitted by each depositor. It also describes about the targets for which the compound is being screened at present.

5.5 Enhancement of the drug like compound library of the Institute

Amit Nargotra, Parvinder Pal Singh and Ram Vishwakarma.

Earlier, the procured drug like compound library of the Institute comprised of 20,000 compounds from Chembridge, which now has been enhanced to 50,000 after an addition of 30,000 compounds from ChemDiv Inc. The selection of compounds from ChemDiv was based upon the stringent medicinal chemistry filters such as removal of undesirable reactive groups, suspicious chemotype and several drug-like physicochemical criteria applied on a library of more than 8 lakh compounds from ChemDiv. The selected compounds were further reduced by removing the duplicates/similar scaffolds from the existing library of 20,000 compounds. As a result the Institutional drug like compounds now contains a diverse set of 50,000 compounds.

6. NATURAL PRODUCT CHEMISTRY (PLANTS)

6.1 Bakuchiol derivatives as novel and potent cytotoxic agents: A report

Rabiya Majeed, M.V. Reddy, Praveen K. Chinthakindi, Payare L. Sangwan, Abid Hamid, Gousia Chashoo, Ajit K. Saxena, S.N. Sharma and Surrinder Koul

In our earlier studies on one of the major chemical constituent of *Psoraleacorylifolia*, namely bakuchiol (1), the moderate antibacterial activity of bakuchiol, for oral anti-pathogenic activity, was successfully modulated through modification on the molecule, and eight fold reduction in MIC against several strains of Gram +ve and Gram -ve bacteria achieved.

Bakuchiol which comprise of styryl moiety in conjunction with a monoterpene (the two together known as meroterpene) has been modulated through phenolic group (2-10), substitution in the aryl part (11-13,28) and isopropylidene group (15-16),and in the monoterpene part (13-27, the styryl derivatives 18-27 prepared by Heck coupling reaction on ethenyl group of bakuchiol. All the compounds 2-27 (Fig. 1) thus preparedwere taken up for bioevaluation study against several human cancer cell lines. Initially the derivatives were screened at 50µM concentration for their ability to induce cytotoxicity in a panel of human cancer cell lines encompassing lung(A-549), breast(MCF-7), prostate(PC-3),cervical (HeLa), leukemia(THP-1), CNS/neuroblastoma(IMR-32), and ovarian (OVCAR-5) cancer cell lines (Table1), taking Mitomycin/Adriamycin/5-FU as the gold standard.

In comparison to the parent molecule bakuchiol 1, which showed growth inhibition in the range of 55-74% against these cell lines, the acetyl derivative 2 showed better inhibition profile (63-82% growth inhibition), and the rest of the homologs (3-9) proved to be far less active, thus, the elongation of the carbon chain did not prove to be a good proposition as seen by the deterrent effect on the activity.

Derivatisation in the aryl part of 1was

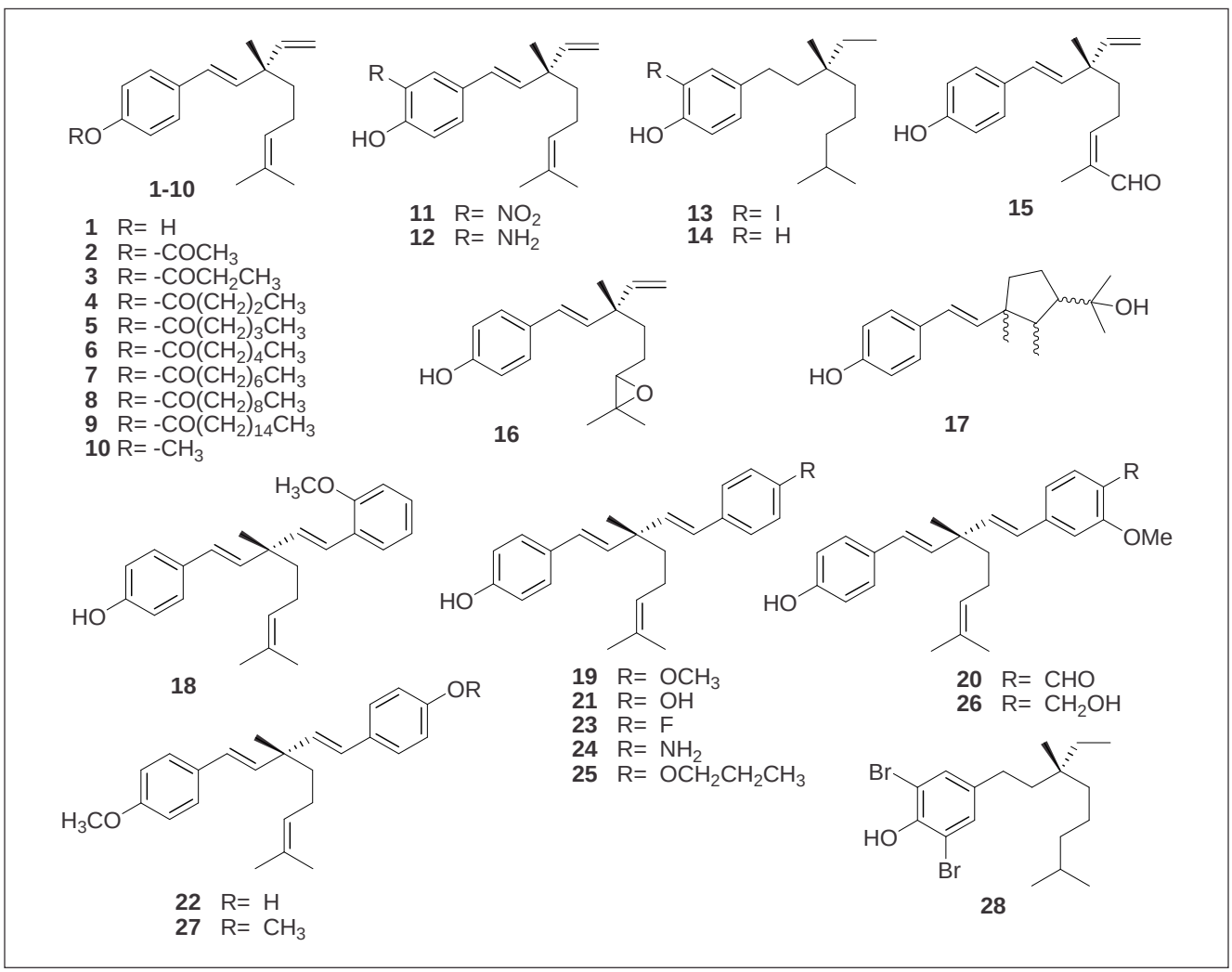


Fig. 1.Structures of Bakuchiol (1) and its analogs (2-28).

carried out to get nitro (11), amino group (12), mono and dihalo derivatives (13, 28,and),and saturated derivative (14) of bakuchiol and these synthetic analogs displayed lower cytotoxicity than the parent molecule1. Modification in the monoterpene part of 1to get derivatives15,16alsodid not help in theenhancement of cytotoxic effect. However, 17, a 1,2,3-trisubstituted cyclopentane derivative obtained as an abnormal oxymercuration-demercuration reaction product of bakuchiol (characterized by spectral analysis),displayed excellent activity profile against leukemia and significant cytotoxicity against all other cell lines under study with 68-93%age growth inhibition.Among the group of bisstyryl derivatives (18-27), compounds 18, 19, 22, & 25proved significant but showed different levels of cytotoxicity which were related to the nature and position of the substituent in the two aryl moieties. The rest of the compounds showed poor activity profiles. It is worth to mention here that limited information is available, related to the cytotoxicity of bisstryryl derivatives.

From SAR point of view, the presence of more than one substituent, in either styryl moiety of the bisstyryls, resulted in the display of low activity (20, 25&26), so did compound 24, having fluoro radical as the substituent, and compound 25 withNH_2 as the substituent (replacing OH group). The racemic compounds 21&27 bearing 4 and 4'-OH groups or 4, and 4' -OMe groups in aryl moieties also showed low inhibitory activity. However, interesting results were observed for two enantiomeric compounds 19 and 22 with *S*-isomer displaying far better activity than the *R*-isomer. While 19(*R*-isomer) showed 50-55 percentage growth inhibition only, its antipode (22) displayed far higher inhibitory effect (68-93% inhibition) against all the cancer cell lines across the board (Table 1), the exhibition of different levels of activity and toxicity associated with enantiomers is well known phenomena.

Table1

Cytotoxic activity* (%age growth inhibition)exhibited by Bakuchiol and its derivatives at 50µM concentration against various human cancer cell lines.

Compounds	Breast MCF-7	Lung A-549	Prostate PC-3	Ovary OVCAR-5	CNS IMR-32	Cervical HeLa	Leukemia THP-1
1	**63±1**	**63±2**	**58±1**	**66±1**	**58±3**	**55±1**	**74±2**
2	**75±2**	**75±3**	**67±2**	**72±1**	**74±3**	**63±1**	**82±1**
9	24±2	**61±3**	**62±3**	**64±2**	**54±1**	**51±2**	**62±2**
11	**49±2**	**52±1**	**52±3**	**55±2**	**50±3**	**49±1**	**55±3**
17	**81±2**	**76±2**	**86±2**	**75±1**	**88±3**	**68±1**	**93±2**
22	**79±2**	**70±2**	**93±1**	**68±1**	**69±2**	**78±2**	**88±2**
5-Fu#	-	**78±2**	**76±1**	**75±3**	**79±2**	**71±3**	**88±3**
Mitomycin#	-	-	**70±1**	-	-	-	-
Adriamycin#	**88±3**	**76±1**	-	-	-	-	-

*Results are Mean ± SD of three separate experiments, conducted in triplicate at the concentration of 50µM. #Concentration of 5-FU = 20µM, Mitomycin = 1µM, Adriamycin = 1µM.

Further, the molecules that exhibited >50% inhibition at 50 µM concentration were screened at lower concentrations i.e. 30 µM, 20,10 and 5 µM so as to identify the best candidates for detailed biological studies.This resulted in the identification of 17 and 22 as the most potent molecules followed by 2. Compound 2 showed upto 57% growth inhibition at 20µM against colon cancer cell line only, while 17&22 showed inhibition at 20µM against all the cell lines across the board. Compound 17 displayed inhibition against four cancer cell lines at 10 µM, and at 5µMconcentration only against one cell line (THP-1). Compound 22 displayed inhibition against three out of eight cancer cell lines at 10 µM concentration while at 5µM concentration; it showed inhibition only against THP-1 cell line (Table 2).

Results are Mean ± SD of three separate experiments, conducted in triplicate at the concentration of 50µM. #Concentration of 5-FU = 20µM, Mitomycin = 1µM, Adriamycin = 1µM.

The IC_{50} value for the three potent cytotoxic compounds 2, 17 and 22 was calculated by non-linear regression analysis using Graph Pad Software (2236 Avenida de la Playa La Jolla, CA 92037, USA). The analogs displayed low IC_{50} values (< 25-2.0 µM) against all the cancer cell lines (Table 4). Since THP-1 was found to be the most sensitive cells towards the cytotoxic potential of these compounds, the IC_{50} value was further calculated at two time points (48 h and 24 h) on HL-60 cells by MTT assay. Both the compound 17 and 22 showed concentration and time dependent inhibition of cell proliferation displaying the IC_{50} values 1.8 µM and 18 µM for 17 while 2.0 µM and 16 µM for 22 after 48 h and 24 h time incubation respectively. Further experiments were carried out to verify whether the cancer cell death induced by the 17 and 22 was apoptotic, and these were observed to be potent apoptosis inducers, as evidenced from the measurement of two important biological end-points of the apoptosis viz., DNA fragmentation and increase in sub-G0 DNA fraction. The apoptotic potential of 17&22 was confirmed through induction of DNA fragmentation in HL-60 cells, which is known as the hallmark of apoptosis (Fig. 2 &3).

($1x10^6$/mL) in culture were treated with indicated conc. of 17 for 24 h.

Table 2
Cytotoxic activity* (%age growth inhibition)of Bakuchiol derivatives at 20µM, 10µM and 5µM concentration against human cancer cell lines.

Compounds	conc. µM	Breast MCF-7	Liver HEP-2	Lung A-549	Prostate DU-145	Leukemia THP-1	Prostrate PC-3	CNS IMR-32	Colon HCT-15
2	20	44±2	46±2	1±2	42±2	50±2	45±3	48±3	**57±2**
	10	31±2	30±1	22±1	29±2	44±2	32±1	34±2	46±1
	5	15±2	14±3	13±2	19±1	34±1	21±2	27±2	31±2
17	20	**50±2**	**50±1**	**53±3**	**54±2**	**76±2**	**57±2**	**62±2**	**63±3**
	10	32±1	34±3	40±2	**50±1**	**65±3**	35±2	**50±1**	**55±3**
	5	27±2	17±2	32±3	32±3	**54±2**	24±4	46±1	29±3
22	20	**50±3**	**58±4**	**69±1**	**73±2**	**78±4**	**58±2**	40±3	**61±3**
	10	37±1	46±1	47±1	**57±1**	**62±1**	41±1	32±1	**50±2**
	5	24±1	23±1	24±1	20±4	**53±2**	23±2	13±1	28±3
5-Fu#	20	-	-	**78±2**	**75±1**	**85±3**	**75±3**	**83±2**	**63±1**
Mitomycin#	1	-	**78±3**	-	-	-	-	-	-
Adriyamycin#	1	**91±1**	-	-	-	-	-	-	-

Table 3
IC_{50} values of selected compounds (2,17,22)against various human cancer cell lines.

Tissue Type	Leukemia		Prostate		Liver	Breast	Lung	CNS	Colon
Cell Lines	THP-1	HL-60	DU-145	PC-3	HEP-2	MCF-7	A-549	SF-295	HCT-15
Compounds IC_{50} in µMs									
17	3.5±1.1	1.8±1.2	14±2	19±1	19±2	22±3	17±3	23±2	13±1
22	4.2±1.3	2±1.1	11±3	15±3	14±2	23±3	12±1	22±3	13±1
2	18±2	ND	26±3	23±2	20±1	25±2	40±3	8±1	11±2

ND= not determined

Cells were stained with PI to determine DNA fluorescence and cell cycle phase distribution by flow cytometry. Fraction of cells for hypo diploid (sub-G0, ≤2n DNA) population indicative of DNA damage was analyzed and shown as (%). Data are representative of one of two similar experiments.

Fig. 3.HL-60 cells ($1x10^6$/mL) in culture were treated with indicated conc. of 22 for 24 h time period. Cells were stained with PI to determine DNA fluorescence and cell cycle phase distribution by flow cytometry. Fraction of cells for hypo diploid (sub-G0, ≤2n DNA) population indicative of DNA damage was analyzed and shown as (%). Data are representative of one of two similar experiments.

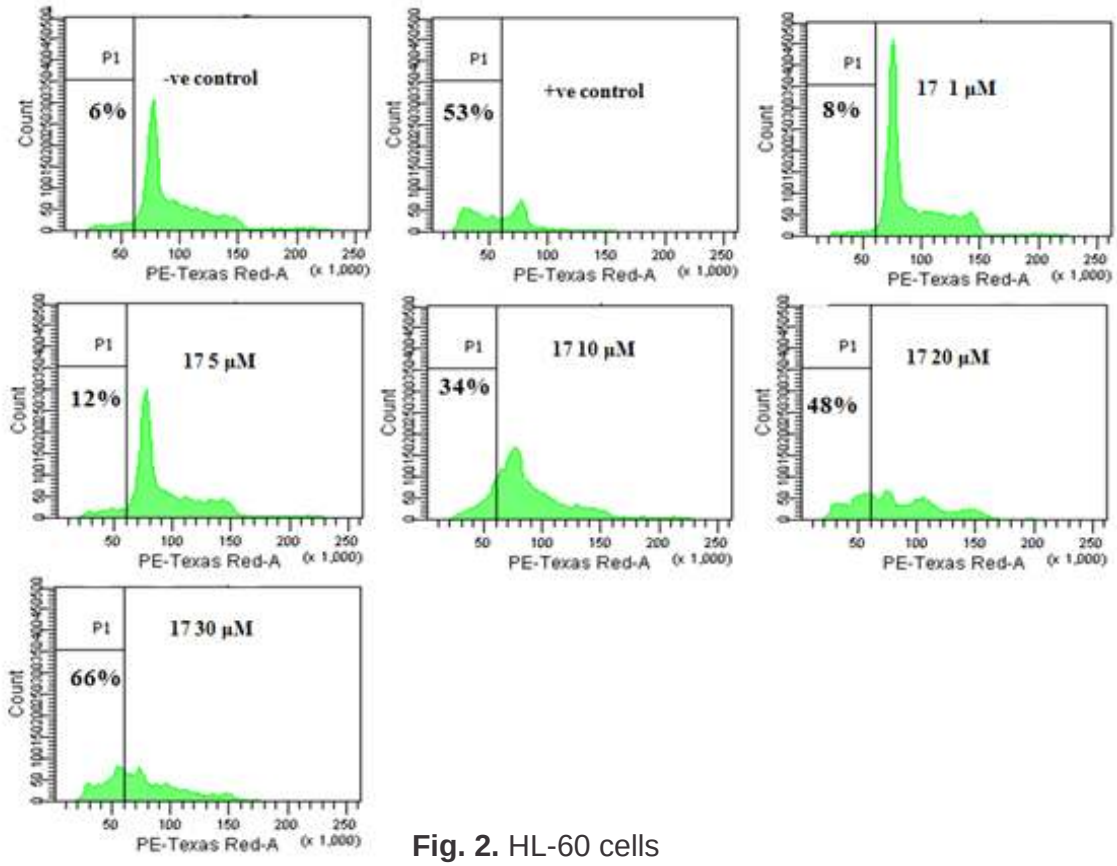
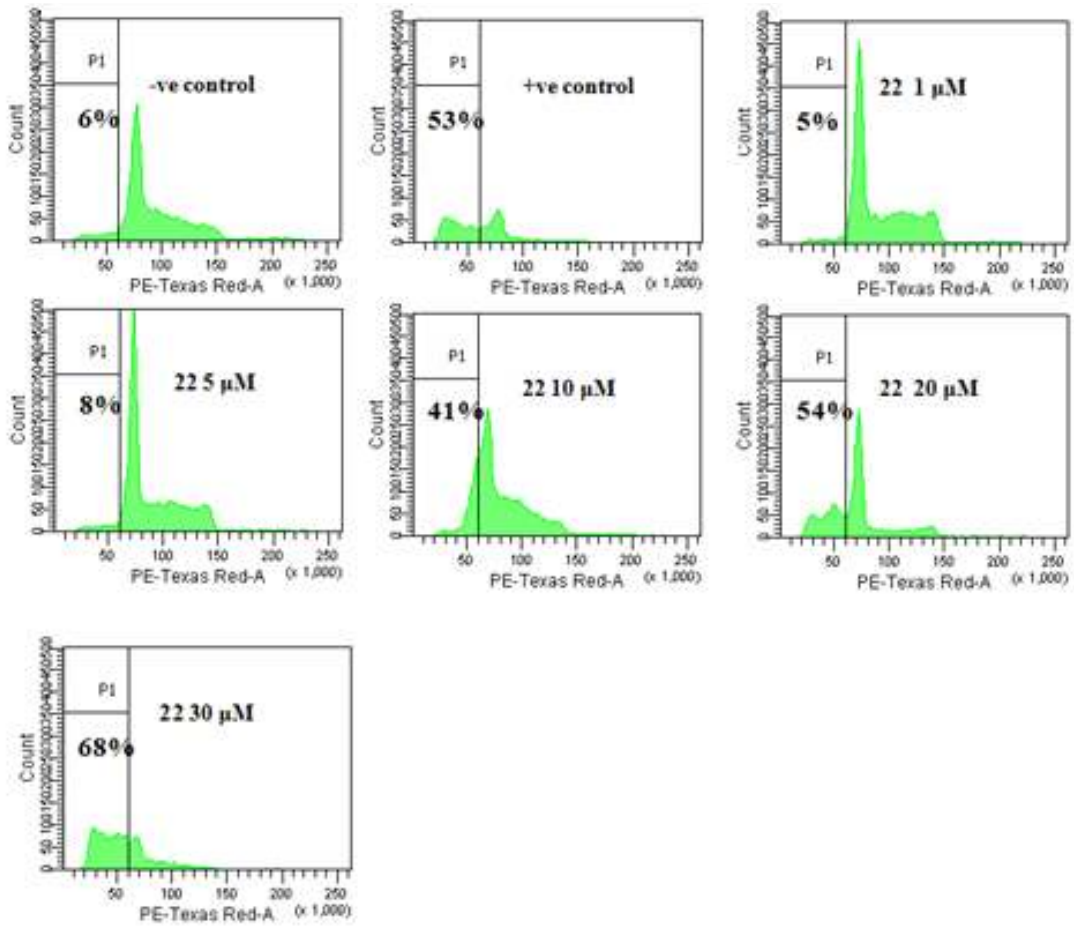


Fig. 2. HL-60 cells

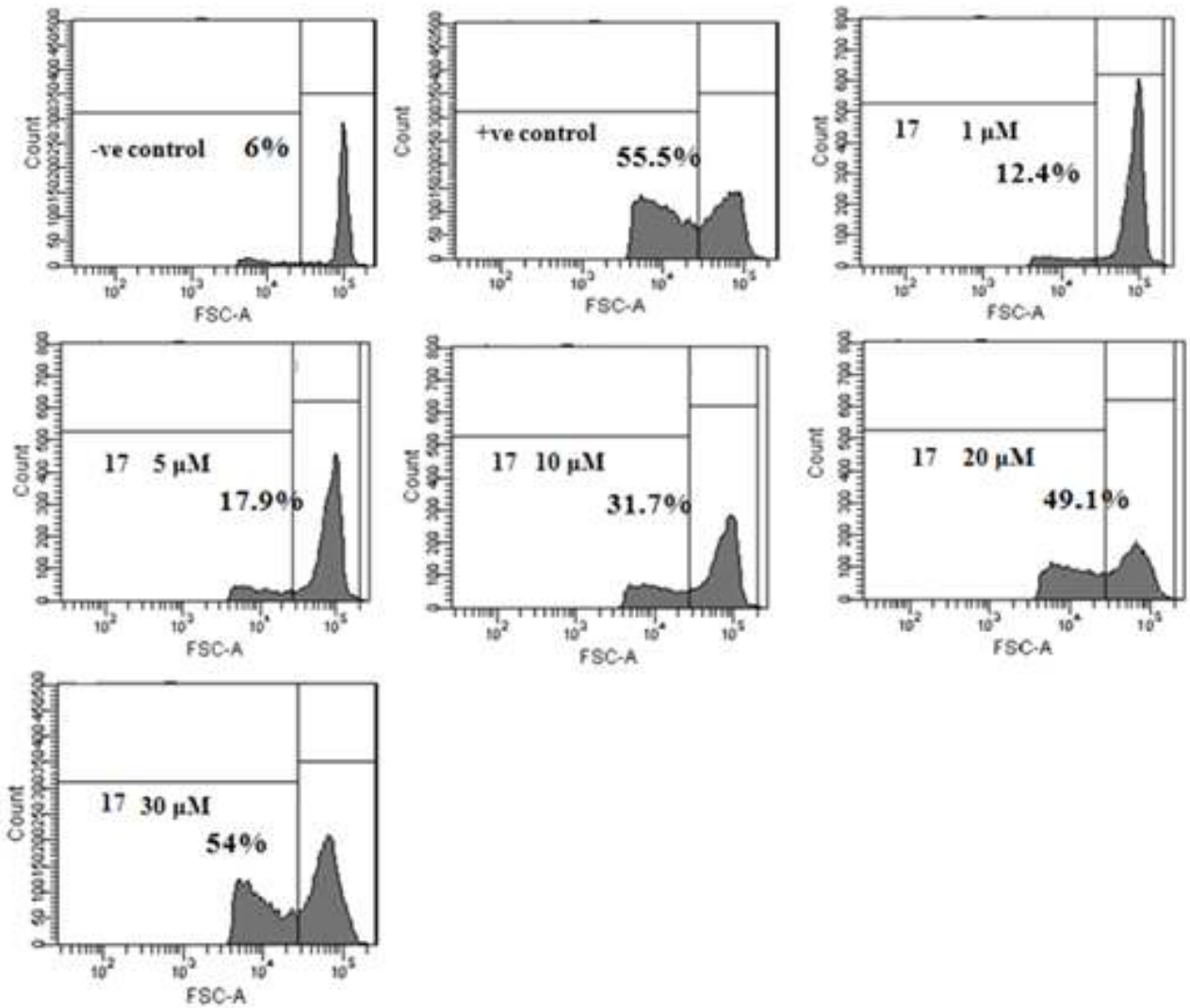


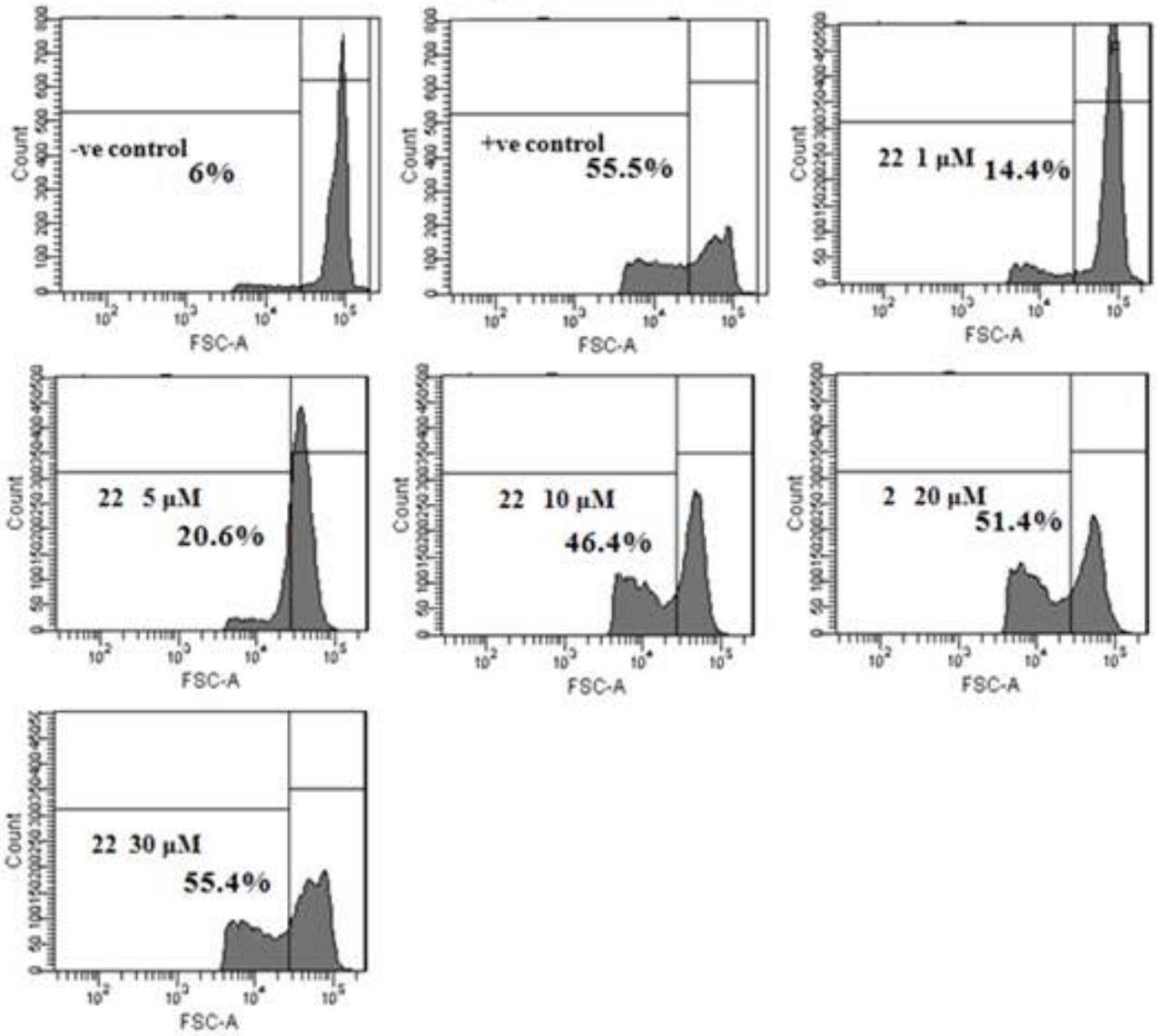
(Fig. 4& 5).Compound 17 showed ~54% of dissipation of mitochondrial membrane potential and 22 showed 55.4%, the untreated control showed only 6% and cells treated with camptothecin showed 55.5% (at 5µM) suggesting the central role of mitochondria towards the apoptotic potential of both of these molecules.

Fig. 4.Compound17induced loss of mitochondrial membrane potential (?Ψ_m) HL-60. Cells ($1x10^6$/mL/ well) incubated with 17 at different conc. in 6 well plates for 24 h. Before 1 h of the completion of the experiment cells were treated with Rodamine-123 (5 µM). Cells were washed with PBS and centrifuged and finally dissolved in 1ml of PBS and analyzed. Data are representative of one of two similar experiments.

Fig. 5. Compound 22induced loss of mitochondrial membrane potential (?Ψ_m) HL-60. Cells ($1x10^6$/mL/ well) incubated with22at different conc. in 6 well plate for 24 h. Before 1 h of the completion of the experiment cells were treated with Rodamine-123 (5µM). Cells were washed with PBS and centrifuged and finally dissolved in 1ml of PBS and analyzed on flow cytometer. Data are representative of one of two similar experiments.

The studies provide the possibility of exploiting bakuchiol based compounds for the development of anticancer therapeutics in leukemia and other cancers.





6.2 Simultaneous quantification of five marker compounds of *Betula utilis* stem bark using a validated HPTLC method

Imran Khan, Payare L. Sangwan, Jagdish Kumar Dhar and Surrinder Koul

Betula utilis D. Don (family Betulaceae),commonly known as Bhojpatra-an important medicinal plant is widely used in Ayurveda and Unani system of medicine, in the treatment of various ailments and diseases such as wound healing, skin disinfectant, bronchitis, convulsions, leprosy and diseases of the blood and the ear. To date, only few analytical methods have been reported for the analysis of the various bioactive ingredients in the birch bark, including betulin, betulinic acid by HPLC and GC–MS.However, the literature shows no report till date about the simultaneous separation of betulin (1), lupeol (2), oleanolic acid (3), oleanolic acid-3-acetate (4) and β-sitosterol (5) as well as their validated quantitative analysis by HPTLC method. HPTLC methodology workable at microgram and nanogram scale requires far less solvent consumption than the HPLC method and has also the advantage of reduction of the run time, cost of analysis. We developed a validated analytical procedure for the simultaneous quantification of isolated marker compounds 1-5 (Fig. 1) from *Betula utilis* stem bark extract using HPTLC method. Various validation aspects of the analysis like as linearity, specificity, recovery, precision, and robustness, limit of detection (LOD) and limit of quantitation (LOQ) have been measured.

The chromatographic conditions were optimized to obtain chromatograms with a good resolution of adjacent peaks. The n-hexane: ethyl acetate (8:2 v/v) system afforded the best separation with distinct $R_f$difference and a successful base line separation of the marker compounds 1-5 (Fig. 2. Segment A) was achieved.The mean

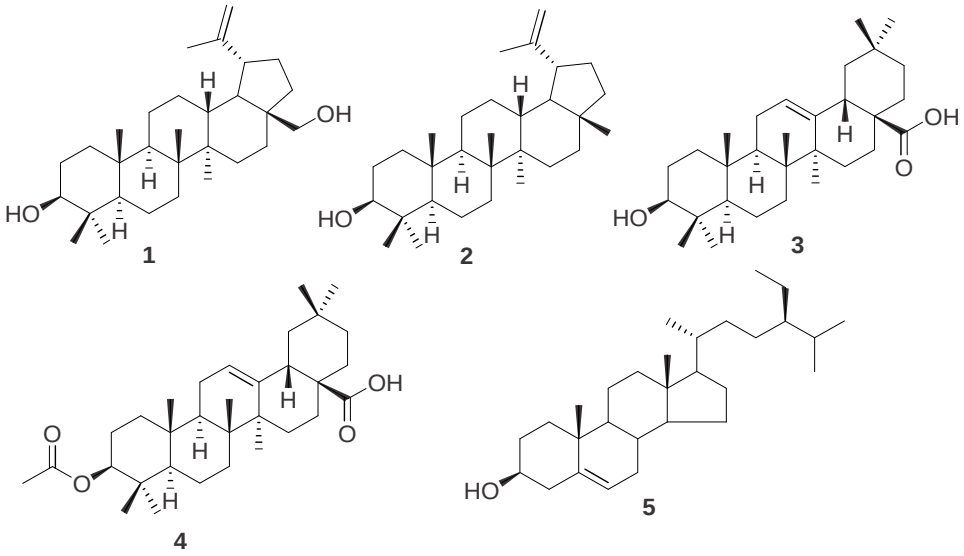
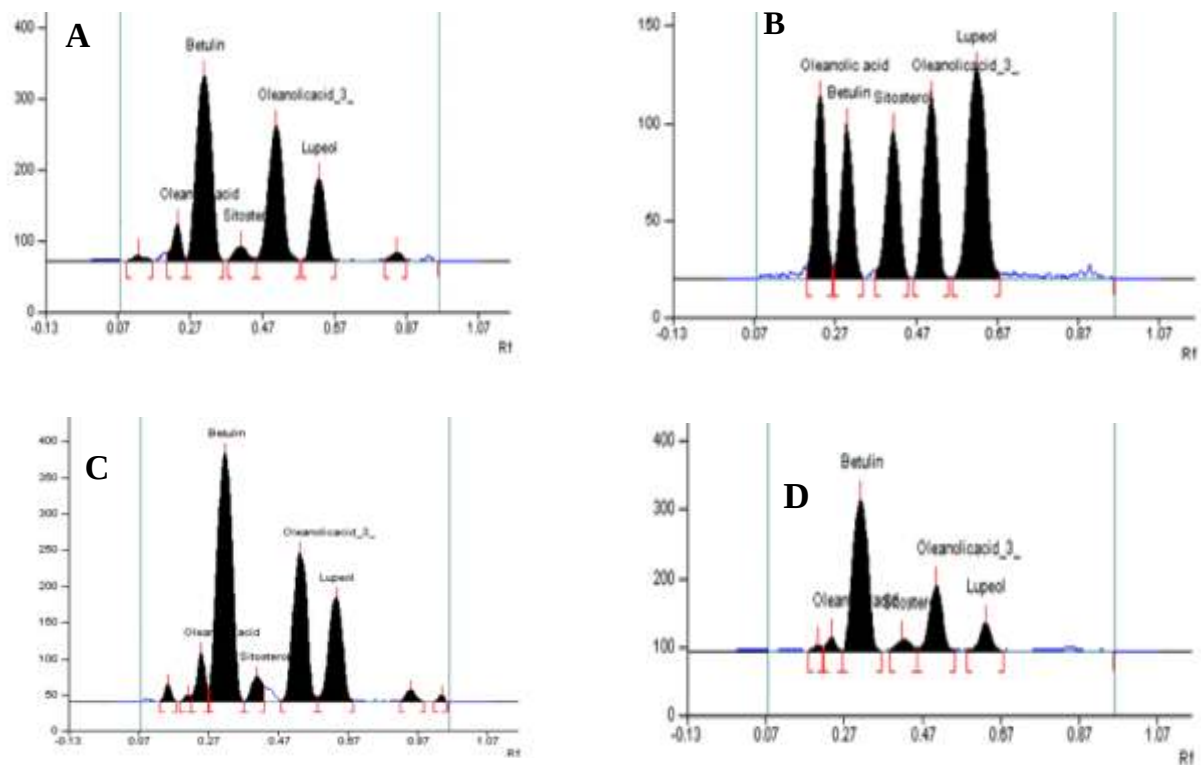
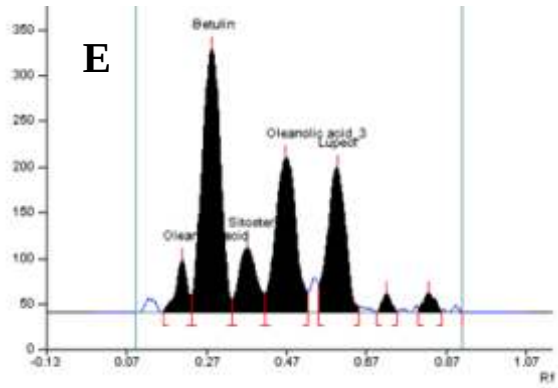


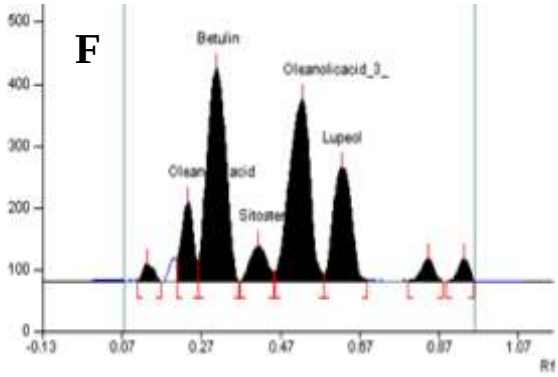
Fig. 1.Structure of marker compounds isolated and quantified in stem bark of *Betula utilis*:Betulin (1),Lupeol (2),Oleanolic acid (3), Oleanolic acid 3-acetate, (4), β-Sitosterol (5).

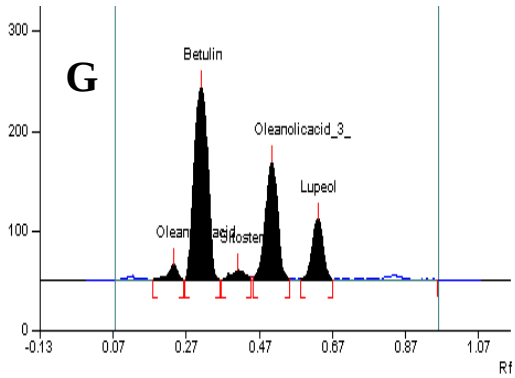
R_f determined for the standards for marker compounds was as follows: betulin 0.31 ± 0.02, lupeol 0.62 ± 0.02, oleanolic acid 0.24 ± 0.01, oleanolic acid-3-acetate 0.51 ± 0.03, and β-sitosterol 0.42 ± 0.02 respectively. The experimental conditions, such as mobile phase composition, scan mode, scan speed and wavelength of detection were optimized to provide accurate and precise results. The developed chromatograms produced compact, flat and dark bands of reference standards when viewed under UV 254 nm, 366 nm and white light after the post chromatographic derivatization. The relationship between the peak areas and the amount of standard applied showed good linearity. For routine analysis 6-point calibration curve was used.

Figure 2. Segment A: HPTLC chromatograms of mixture of standards (1-5) and Segment (B-D): Sample extracts of DCM, DCM: MeOH (1:1) and, MeOH extracts of *Betula utilis*. Segment (E-G): HPTLC spiked chromatograms of DCM, DCM: MeOH (1:1) and, MeOH extracts of *Betula utilis*. Segment (H-I) Represent HPTLC plate of different extractsof *Betula utilis* derivatized by ceric ammonium sulfate reagent. Track assignment for HPTLC plate H and I: 1-4: *Betula utilis* stem bark DCM extract, 5-8: *Betula utilis* stem bark DCM: MeOH(1:1) extract, 9-12: *Betula utilis* stem bark MeOH extract Standard track: Mixture of betulin, lupeol, oleanolic acid, oleanolic acid-3-acetate and β-sitosterol.





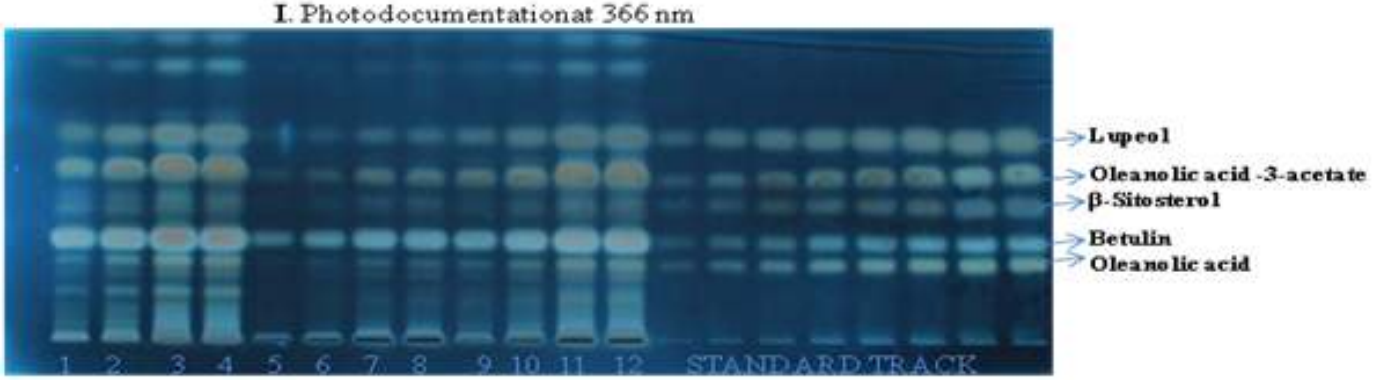




H. Photodocumentation at 254 nm

5
6
3
b
p

I. Photodocumentationat 366 nm



The method validation as per the guidelines of ICH. The goodness offit for all standards was found within the test range of 0.99601–0.99903,and for proving linearity, six calibration pointswere analyzed over the range of 100–600 ng/band for the test samples and repeatability found <5% for three levels (low, medium, and high) of the test samples. The developed method also hadgood accuracy with extraction recovery of 95–99%.

Betulin (1, an important bioactive compound because of its various therapeutic uses and also for the fact that it is the precursor of betulinic acid which is under clinical trial for anti-HIV activity) was found as the major constituents of stem bark of plant Betula utilis (2.88%) followed by oleanolic acid-3-acetate (4), lupeol (2), oleanolic acid (3), and β- sitosterol (5). The quantification results of the five marker compounds in the three extracts of Betula utilis (% w/w) are summarized in Table 1.

Table 1.Content of marker compounds (1-5) (%w/w) in plant material of *Betula utilis* stem bark by HPTLC

Marker compounds	Dichloromethane extract	Dichloromethane: methanol (1:1) extract	Methanol extract
Betulin (**1**)	2.88±0.05	1.36±0.03	0.25±0.002
Lupeol (**2**)	0.40±0.002	0.33±0.002	0.01±0.003
Oleanolic acid (**3**)	0.26±0.001	0.24±0.004	0.01±0.001
Oleanolic acid-3-acetate (**4**)	0.87±0.002	0.63±0.003	0.02±0.004
β-sitosterol (**5**)	0.19±0.002	0.15±0.004	0.008±0.002

Mean ± standard deviation (SD, n = 3)

After the extraction, isolation and characterization of markers from the plant material, their quantitative determination was carried out. On quantification, 1-5and identificationcarried out by comparison of R_f and their UV spectra with those obtained under the same condition after the post chromatographic derivatization by ceric ammonium sulfate reagent.Typical HPTLC chromatograms of reference compounds with samples are shown in Fig. 2.

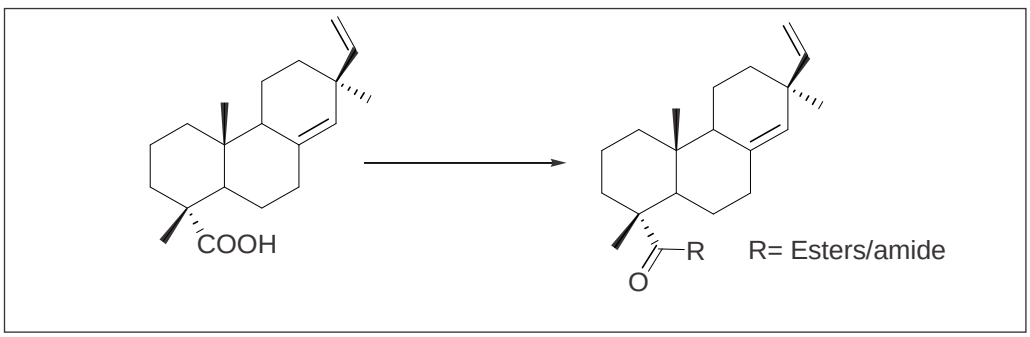
Quantification of the extracts based on these marker compounds has shown betulin as the major constituents with 2.88% natural abundance. The molecule has importance for its therapeutic value being precursor of betulinic acid an anti-HIV molecule under clinical study. HPTLC finger print developed can be helpful in rapid analysis of these phytomolecules in various herbs/herbal formulations/plant products.

The developed HPTLC method was used for the simultaneous quantification of marker compounds

6.3. 4-epi-Pimaric acid a potent antibacterial and anti-biofilam agent for oral cavity pathogens.

F Ali, PL Sangwan, S Koul, A Pandey, S Bani, ST Abdullah, PR Sharma, S Kitchlu and IA Khan

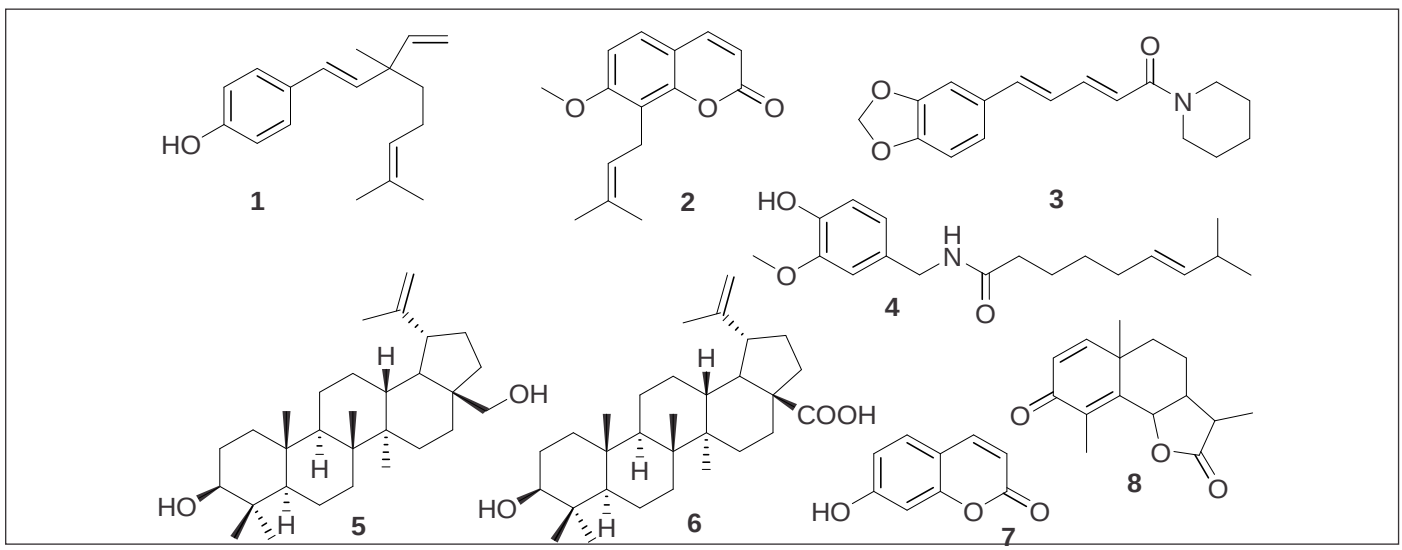
From the ethanol extract of the macerated roots and aerial part of *Aralia cachemirca*, two compounds were isolated and one characterized as 4-*epi*-pimaric acid (1) and the structure of other compound (a diterpene) is being established.On bioevaluation,both the compounds showed antibacterial activity (at 4 and 16 µg/ml respectively) with 4-*epi*-Pimaric acid exhibiting minimum inhibitory concentration (MIC) in the range of 4-16 µg/ml and minimum bactericidal concentration (MBC) two- to four-fold higher than MIC. Its derivatives,an amide and an ester were found bearing less or no activity.



6.4. Natural product chemistry of plants for isolation, characterization of secondary metabolites and their modification.

Alamgir A. Dar, Samar Singh,Nisar A. Dangroo, Saleem Farooq, Praveen K. Chinthakindi, Imran Khan, Rajeshwar R. Aleti, Buddh Singh, R K Thappa, Shanker Lal, Sushma Koul, S Kitchlu, S N Sharma, JL Koul, DK Gupta, PL Sangwan, S Koul

The chemically and pharmacologically unexplored or less explored high altitude plants collected from the different region of Jammu and Kashmir(Project MLP4012) were worked up for the isolation of secondary metabolites following the NCI extraction protocol. One of the major activity being carried out (includes the students pursuing their Ph.D in natural products) in Bioorganic chemistry division involves the isolation of the major (and their chemical modifications) and minor chemical components from these plants. Various class of compoundssuch as xanthones, flavanoids, steroids and terpenes (mainly triterpenes and sesquiterpenes) have been isolated and characterised. Chemical modification of abundantly available major secondary metabolites is also going on to develop structure activity relationship as well as to enhancement in pharmacological activity besides having lesser toxicity or MIC reduction. Some of the selected molecules (Betulinic acid, Bakuchiol, Santonin, Piperine, Osthol, Betulin, Capsaicin,Citral, Scoparone, Boswellic acids) on which modification work is going on are shown in Fig. 1.



Further, work is also going for resolution of isolated marker compounds on high pressure liquid chromatography (HPLC)/ high pressure thin layer chromatography (HPTLC) chromatogram. Finger printing of the extract and fractions and quantification of their markers through validated method development by HPLC/HPTLC.

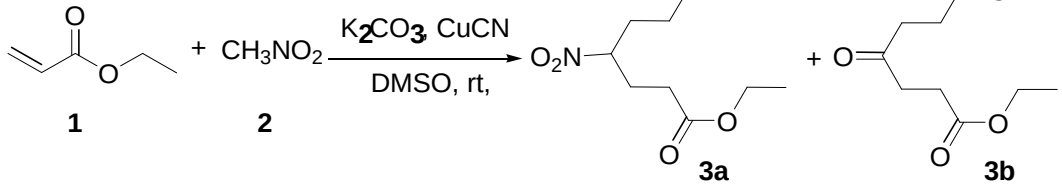
6.5. CuCN catalyzed one pot synthesis of γ-keto diesters: dominodouble Michael addition followed by Nef reaction

Saleem Farooq, Payare L. Sangwan, Rajeshwar R. Aleti, PraveenK. Chinthakindi, Mushtaq A. Qurishi and Surrinder Koul

The spiroketal moiety is a key motif in the plethora of natural compounds present in plants, fungi, insect secretions, shellfish toxin and other living organisms with promising biological activities like antibiotics. Synthesis of such biologically significant cyclic compounds rely on the elaboration of polycarbonyl compounds, among which 1,4-dicarbonyls have proven particularly useful. Though few methodas are available in the literaturefor the synthesis of such functional arrangements, most of these suffer from drawbacks such as requirement of a stoichiometric amount of the Lewis acid, elongated reaction time, hazardous carbon monoxide as source for carbonyl group, and above all expensive and highly toxic catalysts. Surprisingly, apart from these valuable strategies for the generation of γ-keto diesters, there exists no general one pot method available for the synthesis of the said class. Hence, more efficient and practical alternative methods using inexpensive and easily available reagents are warranted. We have developed one pot synthesis of γ-keto diesters in presence of Cs_2CO_3 through CuCN catalyzed domino double Michael reaction followed by Nef reaction to afford the target products, achieved in few minutes, with

moderate to good yields.

The methodology involves the reaction of ethyl acrylate 1 (1 equiv.) and nitro methane 2 (1 equiv.) in DMSO in presence of K_2CO_3 (1.1 equiv.) as a base and CuCN as the catalyst at 20-25 ^{0}C afforded two products (3a and 3b) (Scheme 1) in disproportionate ratio (Table 1), which were isolated in good yields and the products identified by spectral analysis (^{1}H NMR, ^{13}C NMR, IR and MS) and derivatization (reduction of 3a to amino ester and of 3b to hydroxy ester). Prolonged reaction time did not result in any incremental increase of 3b content even and in the absence of CuCN, only Michael reaction product 3a was obtained, and .



Scheme 1 Reaction of ethyl acrylate and nitro methanein the absence of the catalyst, the nitro intermediate failed to afford 3b.In a bid to switch over the reaction more predominantly towards the production of 3b due to its biological and synthetic importance as described earlier, the reaction conditions were optimized in terms of solvent, base and temperature ,catalyst selection which resulted in the improvement in yields, time minimization, and predominance of 3b over 3ashown in tables 1-2.

Table 1

Reaction of ethyl acrylate and nitro methane with CuCN as the catalyst at different temperature in presence of different bases in DMSO and DCM solvents.

S.No.	Base	Temp. (ºC)	Time (h)	Ratio[a] of **3a**:**3b** (combined isolated % yields) in solvent DMSO	DCM
1	K_2CO_3	0-5	6	38:62 (85)	22:68 (86)
		20-25	2	33:67 (87)	28:72 (90)
		30-32	0.5	27:73 (87)	25: 75(89)
2	DBU	0-5	6	47:53 (80)	44:56 (83)
		20-25	2	45:55 (85)	41:69 (86)
		30-32	0.5	39:61 (83)	32:68 (85)
3	DABCO	0-5	6	42:58 (81)	39:61 (83)
		20-25	2	40:60(85)	35:65 (86)
		30-32	0.5	34:66 (83)	30:70 (85)
4	Et_3N	0-5	6	49:51 (80)	40:60 (83)
		20-25	2	45:55 (85)	40:60 (86)
		30-32	0.5	38:62 (85)	32:68 (87)
5	Cs_2CO_3	0-5	6	32:68 (87)	29:71 (89)
		20-25	2	30:70 (89)	24:76 (90)
		30-32	0.5	22:78 (88)	16:84 (90)
6	Pyridine	0-5	6	Exclusive product **3a** obtained	
		20-25	2	(80-83)	
		30-32	0.5		
7	Piperidine	0-5	6	Exclusive product **3a** obtained	
		20-25	2	(82-85)	
		30-32	0.5		
8	Pyrrolidine	0-5	6	Exclusive product **3a** obtained	
		20-25	2	(82-85)	
		30-32	0.5		

Table 2
Reaction of ethyl acrylate and nitro methane in different solvents with Cs_2CO_3 as the base, and CuCN as catalyst.

S.No.	Solvent	Temp. (°C)	Time (h)	Ratio[a] of **3a:3b**	Isolated Yields[b] (%)
1	DMSO	30-32	0.5	22:78	88
2	DCM	30-32	0.5	16:84	90
3	DMF	30-32	0.5	34:66	86
4	CH_3CN	30-32	0.5	35:65	86
5	THF	30-32	0.5	39:61	85
6	CH_3OH	30-32	0.5	Noreaction	--
7	H_2O	30-32	0.5	No reaction	--
8	Ethylene glycol	30-32	0.5	No reaction	--

A ratio of products calculated based on isolated yields.[b]Combined yield of pure isolated products.

Further optimization study was carried out using different copper salt and the best results were obtained with CuCN catalyst (Table 3).

Table 3

Study of different Cu-salts on time, space and yield of the reaction

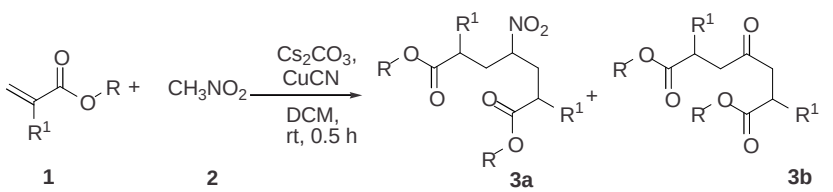
S.No	catalyst	Ratio of **3a:3b** in DCM, with base $K_2CO_3Cs_2CO_3$		Ratio of **3a**:**3b** in DMSO, with base $K_2CO_3Cs_2CO_3$	
1	CuCN	25:75	16:84	27:73	22:78
2	CuCl	31:69	26:74	36:64	30:70
3	CuI	28:72	24:76	30:70	27:73
4	$Cu(CN)_2$	Exclusive product **3a** obtained			
5	$CuCl_2$	Exclusive product **3a** obtained			
6	CuI_2	Exclusive product **3a** obtained			

Reaction time: 0.5 h, Temp: 30-32 °C

Further reactions were carried out to validate the feasibility of the methodology using six different acrylates and subjecting them to react with nitro methane (Scheme 2) and products of ethyl acrylate and nitro methane.

A ratio of products calculated based on isolated yields.[b]Combined yield of pure

nitro ethane (Scheme 3) under the optimized reaction conditions of temperature (30-32 °C), base (Cs_2CO_3), solvent (DCM) using CuCN as the catalyst.



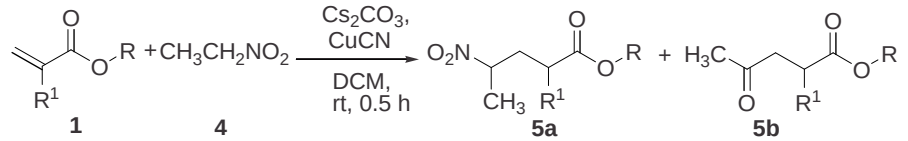
1 **2** **3a** **3b**

1 R^1 =H, R =C_2H_5
1a R^1=H, R =CH_3
1b R^1 =H, R =n-C_4H_9
1c R^1 =CH_3, R =C_2H_5
1d R^1 =CH_3, R =Me
1e R^1 =CH_3, R =n-C_4H_9

3a R^1 =H, R =C_2H_5
3a-I R^1 =H, R =CH_3
3a-II R^1 =H, R =n-C_4H_9
3a-III R^1 =CH_3, R =C_2H_5
3a-IV R^1 =CH_3, R =Me
3a-V R^1 =CH_3, R =n-C_4H_9

3b R^1 =H, R =C_2H_5
3b-I R^1 =H, R =CH_3
3b-II R^1 =H, R =n-C_4H_9
3b-III R^1 =CH_3, R =C_2H_5
3b-IV R^1 =CH_3, R =Me
3b-V R^1 =CH_3, R =n-C_4H_9

Scheme 2 Reaction of different alkyl acrylates and CH_3NO_2.



1 **4** **5a** **5b**

1 R^1 =H, R =C_2H_5

1a R^1 =H, R =CH_3

1b R^1 =H, R =n-C_4H_9

1c R^1 =CH_3, R =C_2H_5

1d R^1 =CH_3, R =Me

1e R^1 =CH_3, R =n-C_4H_9

5a R^1 =H, R =C_2H_5

5a-I R^1 =H, R =CH_3

5a-II R^1 =H, R =n-C_4H_9

5a-III R^1 =CH_3, R =C_2H_5

5a-IV R^1 =CH_3, R =Me

5a-V R^1 =CH_3, R =n-C_4H_9

5b R^1 =H, R =C_2H_5

5b-I R^1 =H, R =CH_3

5b-II R^1 =H, R =n-C_4H_9

5b-III R^1 =CH_3, R =C_2H_5

5b-IV R^1 =CH_3, R =Me

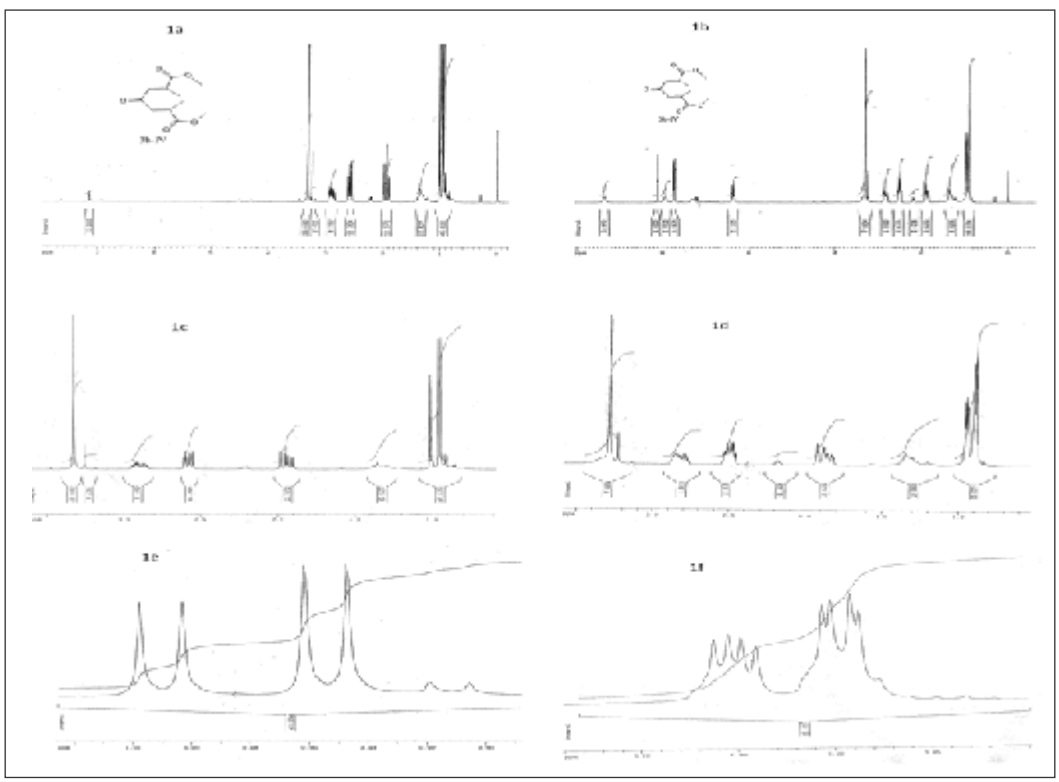
5b-V R^1 =CH_3, R =n-C_4H_9

Scheme 3 Reaction of different alkyl acrylates and nitro ethane

The reactions proceeded smoothly with good to excellent overall yields and with substituted acrylates (Table 4), the desired product was obtained as single entity (diastereoisomeric ratio: >99%, confirmed by spectral analysis, entries 3b- III-V and 5a-III-V). The proton spectrum of compound 3b IV on addition of CSA [S(+)-1-(9-anthryl)—2,2,2-triflouro-ethanol] showed fine splitting of methyl and methine proton signals (Fig. 1) leading thereby to conclude that the diastereoisomer is apparently a racemic mixture rather than a meso compound. The splitting pattern of proton signals was also

S.No.	Substrate	Reaction with CH_3NO_2		Reaction with $C_2H_5NO_2$	
		3a:**3b** Ratio	yield (%) combined	**5a**:**5b** Ratio	yield (%) combined
1	Ethyl acrylate	16:84	83-90	22:78	85-88
2	Methyl acrylate	19:79	80-85	26:74	80-83
3	Butyl acrylate	28:72	70 -72	30:70	67-70
4	Methyl methacrylate	27:73	69-70	25:75	67-73
5	Ethyl methacrylate	29:71	65-70	22:68	65-68
6	Butyl methacrylate	30:70	66-70	32:68	66-68

Reaction time: 0.5 h, Temp: 30-32 °C



observed for 5a- IV.

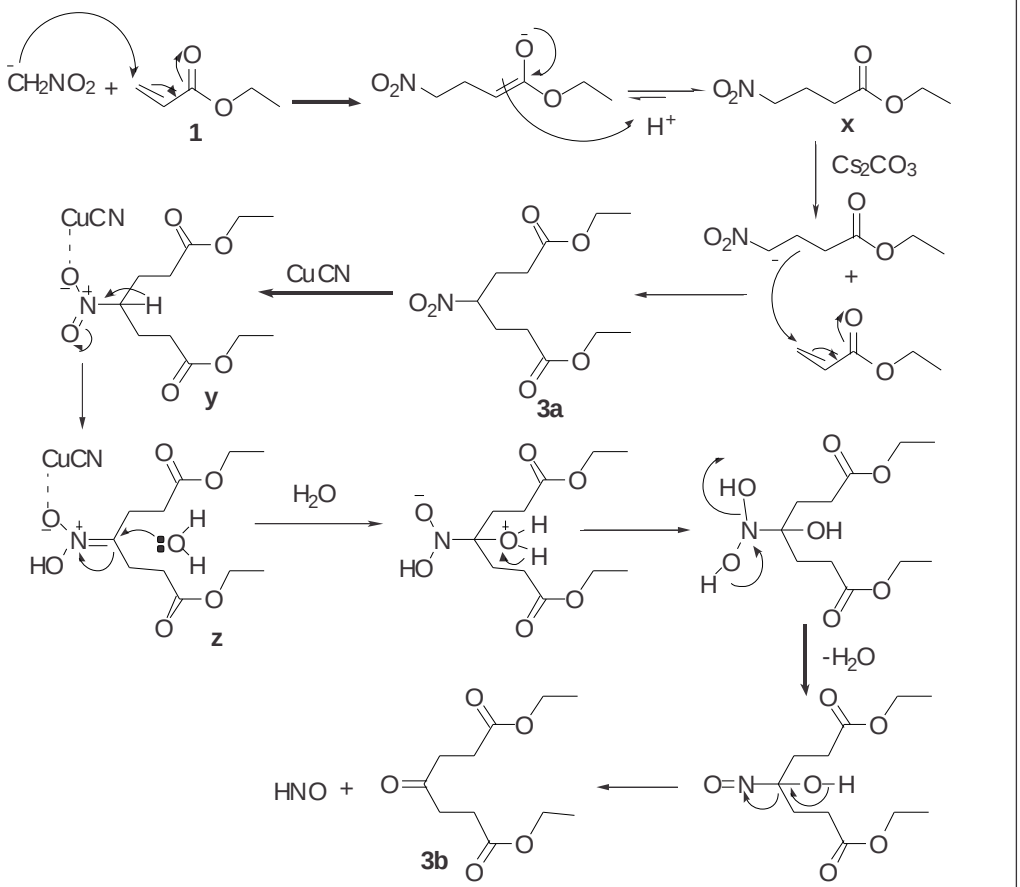
Table 4

Reaction of different alkyl acrylates with CH_3NO_2 and $C_2H_5NO_2$ in presence of CuCN catalyst, Cs_2CO_3 base in DCM.

Figure 1.^{1}H NMR specta of 3b- IV in the absence and presence of CSA. 1a: Specta in absence of CSA and 1c and 1e its expanded spectra. 1b: Specta in presence of CSA and 1d and 1f its expanded spectra.

Next, we tried to expand the scope of the reaction on other α,β-unsaturated systems such as citral, acrolein, carvone, and cinnamaldehyde. However, all these substrates failed to furnish the desired products. These results lead to imply that the ester group of the acrylates apparently seems to contribute for the reaction to click.

From mechanistic point of view, account for the formation of the keto diester, a plausible mechanism has been proposed as shown in Scheme 4. The mechanism involves the attack of the carbanion ($:CH_2NO_2$) on the ethyl acrylate under basic conditions to give Michael product "x" (a carbanion) which attacks the second ethyl acrylate molecule to give 4-nitro diester (3a). The nitro group of the latter forms a complex with CuCN which undergoes deprotonation of C-4 to form" y", followed by attack of water molecule to form intermediate "z" and subsequent expulsion of HNO group to result in the formation of keto diester 3b.



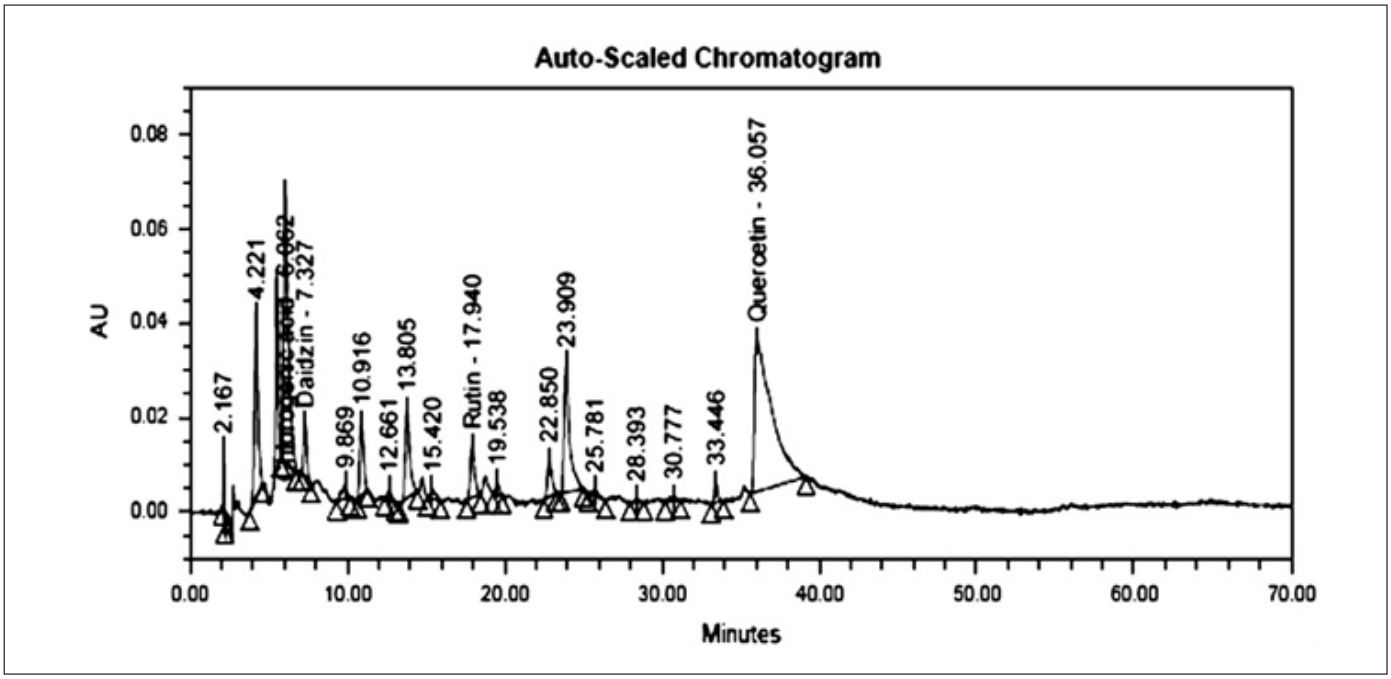
Scheme 4 Proposed mechanism

In conclusion, we have developed a copper catalyzed one pot synthesis of γ-keto diesters using simple available substrates in desirable yields. The procedure described is simple and involves relatively mild reaction conditions.

6. 6 Immunomodulatory studies of a bioactive fraction from the fruit of *Prunus cerasus* in BALB/c mice

Sheikh Abid, Anamika Khajuria, Qazi Parvaiz, Tabasum Sidiq, Aruna Bhatia, Surjeet Singh, Shabir Ahmad, M.K. Randhawa, N.K. Satti and Prabhu Dutt

In order to evaluate the role of ethyl acetate fraction (PNRS-EtOAC) obtained from the Prunus cerasus fruit in the modulation of immune responses, detailed studies were carried out using a panel of in vivo assays. Oral administration of PNRS-EtOAC (25–100 mg/kg) stimulated the IgM and IgG titre expressed in the form ofhemagglutinationantibody (HA) titre. Further, it elicited a dose related increase in the delayed type hyper-sensitivity reaction (DTH) after 24 and 48 h in BALB/c mice. Besides augmenting the humoral and cell mediated immune response, the concentration of cytokines (IFN-γ, IL-4, and TNF-α) in serum with respect to T cell interactions, i.e. the proliferation of lymphocytes were significantly increased at 50 mg/kg compared with the control. The results in these studies demonstrated the



immunostimulatory effect of PNRS-EtOACin a dose-dependent manner with respect to the macrophage activation possibly expressing the phagocytosis and nitrite production by the enhancement of TNF-α production as a mode of action.

Fig.2.Chromatogram of standard mixture of four compounds. The HPLC analysis of the pure compounds was performed at a flow rate of 1.0 mL/min using mobile phase consisting of 0.05% TFA in ACN: 0.05% TFA in water (gradient). The photodiode array detector was set at wavelength of 340 nm.

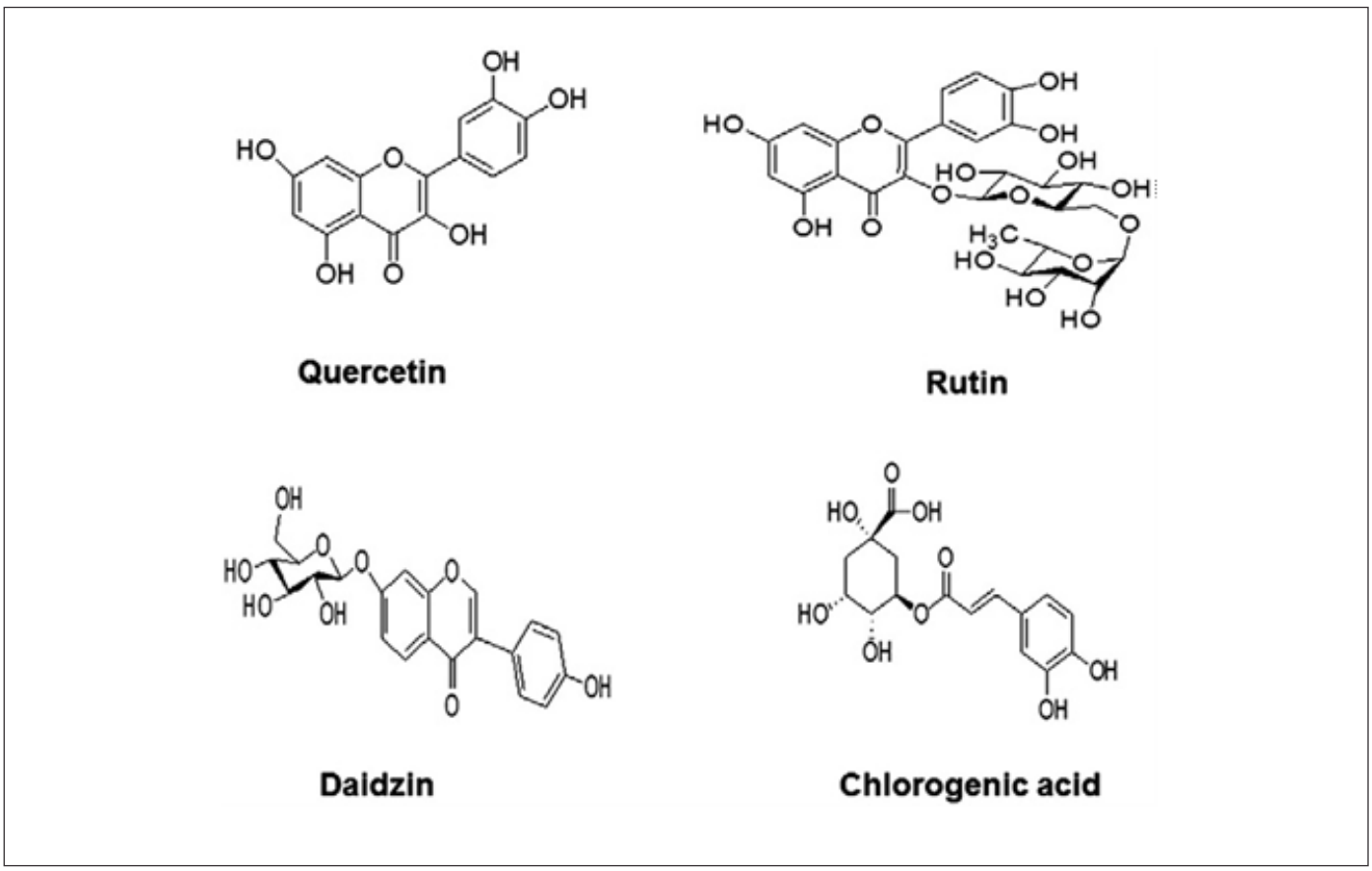
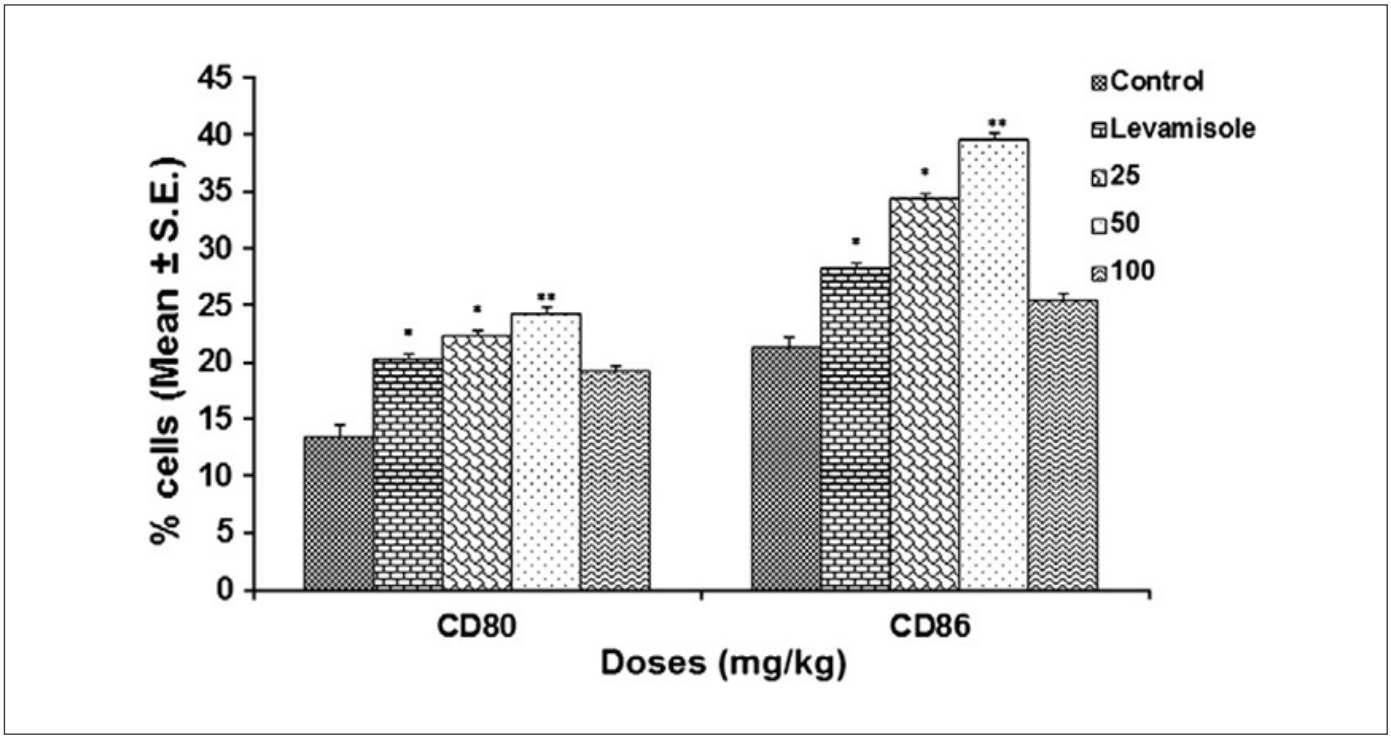


Fig.3. Structures of isolated compounds from PNRS-EtOAC.

The working solutions of allthe marker compounds were mixed together in the ratio (chlorogenic acid:daidzin:rutin:quercitin: 2:4:3:1) and were injected in different concentrations (5 µL, 10 µL,15 µL, 20 µL, 25 µL). The calibration curve of each marker compound in the mixturewas plotted using five levels of concentrations and linearity of each marker compoundwas observed in the concentration range 48 µg–240 µg for chlorogenic acid, 320 µg–1600 µg for



daidzin, 60 μg–300 μg for rutin and 64 μg–320 μg for quercitin. The markercompounds in the ethyl acetate fraction were quantified using these calibration curves.

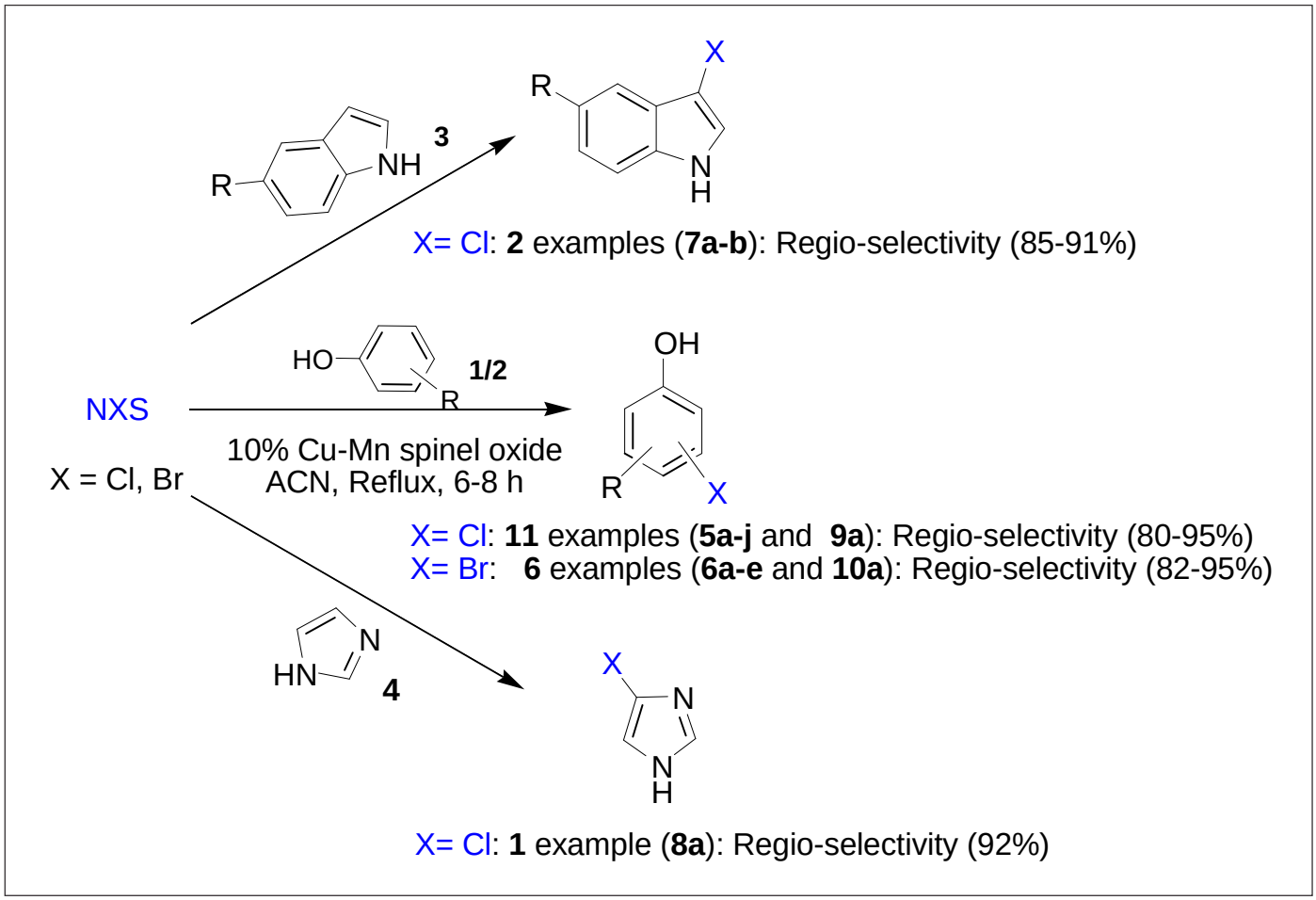
Fig. 10. Flow cytometric analysis of the expression of co-stimulatory signal molecules in spleen-derived macrophages. To quantify the expression of co-stimulatory molecules, 2 × 106 macrophages were stained with FITC-labeled anti-CD80 (B7-1) and anti-CD86 (B7-2) mAbs. Other conditions were the same as described in Materials and methods section. Data represented by percent CD80/CD86 positive cell populations are mean ± S.E. of six animals. *Pb0.05 and **Pb0.01 compared with control group determined by one-way ANOVA (Bonferroni correction multiple comparison test).

It is clear from this study that PNRS-EtOAC played an importantrole in the modulation of the immune response and thus may haveapplications in combating various life-threatening infections. Therefore, it could be a drug of choice, effective in treating the diseaseswhere the underlying defect is a T-cell and B-cell deficiency or phagocytic dysfunction.

7. MEDICINAL CHEMISTRY

7.1. Cu-Mn spinel oxide catalyzed regio-selective halogenation of phenols and N-heteroarenes

Parvinder Pal Singh, Thanusha Thatikonda, K. A. Aravinda Kumar, Sanghapal D. Sawant, Baldev Singh, Amit Kumar Sharma, P. R. Sharma, Deepika Singh and Ram A. Vishwakarma



A novel simple, mild chemo-and regio-selective method has been developed for the halogenation of phenols using Cu-Mn spinel oxide as a catalyst and N-halosuccinimide as halogenating agent. In the presence of Cu-Mn spinel oxide B, EWG- and EDG-bearing phenols as well as N-containing heteroarenes *viz*., indole and imidazole gave mono-halogenated product in good to excellent yields with highest *para*-selectivity. The *para*-substituted phenol also gave mono-halogenated product with good yield and *ortho*-selectivity. Unlikely copper catalyzed halogenation of phenols, present method works well with EWG-bearing phenols and gave comparatively better yields and selectivity. The Cu-Mn spinel catalyst is robust and reused three times under optimized conditions without any loss in catalytic activity. Non-phenolics did not undergo this transformation.

In summary, a new method for regio-selective mono chlorination/bromination of phenols and N-heteroarenes in the presence of robust and reusable bimetallic Cu-Mn spinel oxide catalyst using simple and inexpensive N-halosuccinimides as halogenating agent is established. The present method is very general and is applicable to both EDG- and EWG-bearing phenols. Moreover, in the present method EWG-bearing phenols gave comparatively better results in terms of yield and selectivity. The present method is also suitable for various N-heteroarenes such as indole and imidazole. These transformations provide regio-selectively for chlorination and bromination to a wide array of phenols and N-heteroarenes. Further exploration for full scope of these reactions and its extension to other arenes and heteroarenes as well as mechanistic study is underway and will be reported in due course.

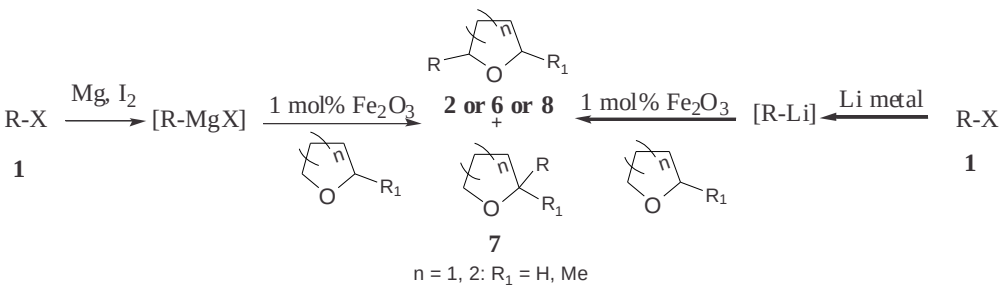
Table 1: Screening of different copper based catalysts for regio-selective halogenations

Entry	Catalyst	Qtyb	Temp (oC)	Time (h)	Product compositiona(%)			
					S	4-Cl	2-Cl	2,4-DiCl
a	Cu-Mn (A)	10c	80	10	26	60	10	3
b	Cu-Mn (B)	10c	80	10	7	70	3	12
c	Cu-Mn (C)	10c	80	10	25	61	8	3
d	Cu-Mn (B)	10d	80	10	3	70	2	21
e	Cu-Mn (B)	10e	80	08	9	80	2	3
f	Cu-Mn (B)	20e	80	10	24	54	9	9
g	Cu-Mn (B)	5e	80	10	13	68	6	8
h	Cu-Mn (B)	1e	80	10	28	53	5	10
i	-		80	10	20	45	33	-
j	Cu-Mn (B)	10e	rt	36	33	40	27	-
k	-		rt	36	35	42	23	-
l	CuI	10e	80	10	17	43	32	-
m	Cu_2O	10e	80	10	18	44	34	-
n	$Cu(OAc)_2$	10e	80	10	50	25	20	-
o	$CuCl_2$	10e	80	10	33	49	13	4

a The products were characterized by GC-MS; bCatalyst wt% equiv w.r.t. phenol.
c Reaction condition: Phenol (1 mmol) and NCS (1 mmol) in ACN.
d Reaction condition: Phenol (1 mmol) and NCS (1.2 mmol) in ACN
e Reaction condition: Phenol (1 mmol) and NCS (0.9 mmol) in ACN.
The number in the product composition denote the position of chlorination w.r.t. -OH group.
S refers to the substrate and Cu-Mn (A) =(Cu:Mn:2:0.25); Cu-Mn (B) = (Cu:Mn:1:0.25); Cu-Mn (C) = (Cu:Mn:3:0.25)

7.2. New method for C-H arylation/alkylation at α-position of cyclic aliphatic ethers by iron-oxide mediated reaction

Parvinder Pal Singh, Satish Gudup, Hariprasad Aruri, Umed Singh, Srinivas Ambala, Mahipal Yadav, Sanghapal D. Sawant and Ram A. Vishwakarma



Iron oxide mediated C-C bond formation

A new and efficient iron oxide catalyzed cross-coupling reaction is established between organometallic species such as alkyl/arylmagnesium halides or organolithium species and α-hydrogen bearing cyclic unbranched and branched aliphatic ethers via activation of $C(sp^3)$-H. In the presence of 1mol% of iron oxide, five and six membered unbranched cyclic ethers such as tetrahydrofuran and tetrahydropyran gave good to excellent yields of cross-coupled products. Whereas, in case of branched ether such as 2-methyltetrahydrofuran, it was observed that the arylation occured at both the sides and gave moderate yields of mixture of regio-isomers. Among the organometallic species used, alkyl organometallic reagents gave less yields as compared to aryl organometallics. Iron oxide mediated direct C-C bond formation without expensive or toxic ligands. In

Scheme-1: Iron oxide catalyzed cross coupling reaction of organometallic species with THF via activation of a-$C(sp^3)$-H

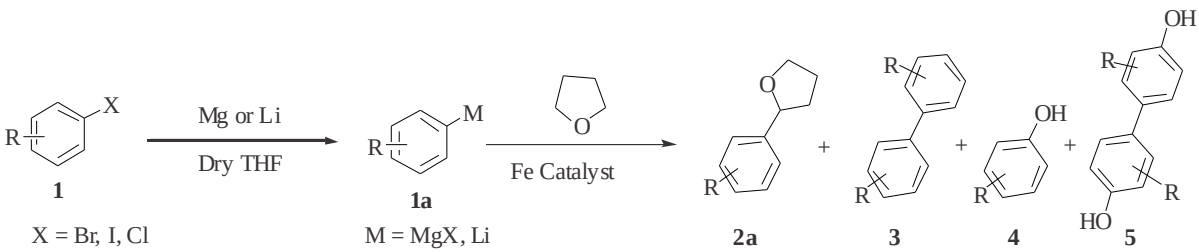
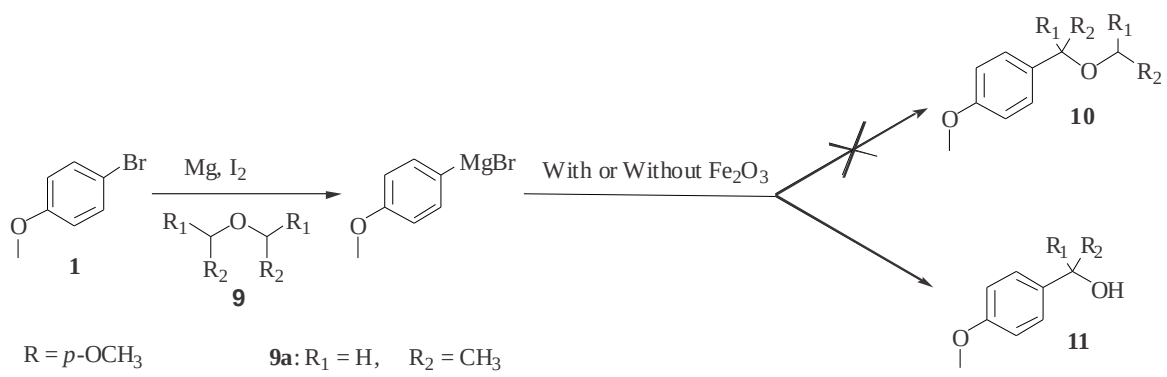


Table 1:-Screening of different iron based catalysts

Entry	Catalyst	Catalyst Qty.	Temp.	Time (h)	Yields (%)[a]			
					2a	**3**	**4**	**5**
a	$Fe(OH)_3$	10 mol %	rt	8	40	10	20	5
b	$FeCl_3$	10 mol %	rt	8	<5	8	-	-
c	Fe_2O_3	10 mol %	rt	8	77	4	7	1
d	Fe_2O_3	10 mol %	0 °C	8	85	3	1	-
e	Fe_2O_3	10 mol %	-10 °C	12	92	2	1	-
f	Fe_2O_3	10 mol %	-70 °C	12	90	2	1	-
g	Fe_2O_3[a]	10 mol %	-10 °C	8	85	5	8	-
h	**Fe_2O_3**	**1 mol %**	**0 °C**	8	**95**	-	0.5	-

[a,] HPLC yield.

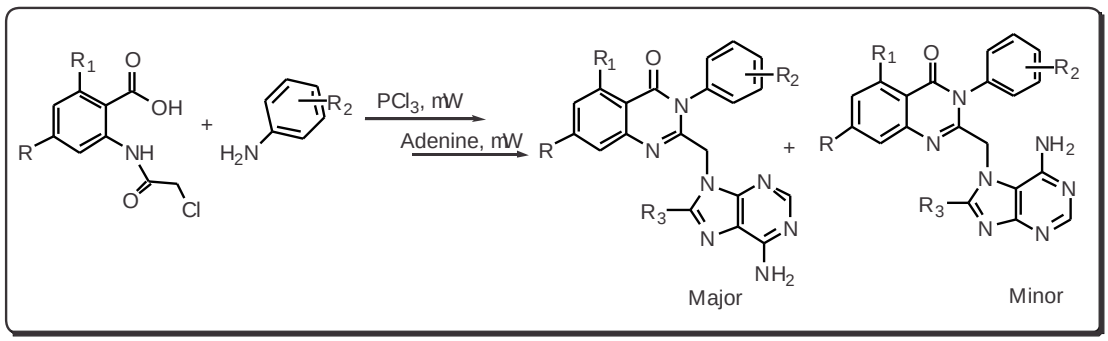
Scheme 2: Studies towards the cross coupling of arylmagnesium bromide with various acyclic ethers



summary, we have discovered a new method for the cross-coupling of organometallic species with cyclic unbranched and branched aliphatic ethers*via* activation of α-C(sp^3)-H bond using Fe_2O_3. This Fe_2O_3 catalysed cross coupling reaction is very selective towards cyclic ethers. No toxic or/and expensive ligand is required for this metallic catalysis. Further studies on the mechanism of this new method as well as attempt towards the regioselective/stereoselectivity of this reaction are currently underway.

7.3. One pot multicomponent synthesis of medicinally important purine quinazolinone derivatives

Sanghapal D. Sawant, Mahesuni Srinivas, G. Lakshma Reddy, V. Venkateswar Rao, Parvinder Pal Singh and Ram A. Vishwakarma



We have developed an efficient one pot synthesis of purine quinazolinone derivatives. The present protocol offers microwave assisted multiple-component one pot synthetic strategy for the construction of the medicinally important purine quinazolinone scaffold. Series of compounds are prepared by cyclization and condensation reactions using this approach. The compounds are structural analogs of anticancer agents IC-87114 and CAL-101, which are highly isoform selective PI3K-δ inhibitors and are presently under clinical investigation for chronic lymphocytic leukemia.

The present protocol offers an environmentally friendly and

Table 1: Optimization of reaction conditions for synthesis of 5.

Entry	Substrate	MW (Watt)	Reaction time (min)	Product yields[a] (%): **5**
1.	3	100	3	45
		250	3	78
		350	3	>95
		450	3	Decomposed product

[a]Isolated yields

Table 2: Optimization of the reaction conditions for the synthesis of 7 using intermediate compound 5

Entry	Substrate	MW (Watt)	Temperature	Reaction time (min)	Product yields[a] (%)	
					7a	
a.	5	50	50 °C	3	35	8
				5	38	8
				7	40	7
			100 °C	3	50	10
				5	52	9
				7	52	10
			150 °C	3	56	12
				5	58	12
				7	59	13
b.	**5**	**100**	50 °C	3	62	13
				5	64	14
				7	64	13
			100 °C	3	70	17
				5	**80**	**20**
				7	74	17
			150 °C	3	67	15
				5	69	15
				7	65	14

c.	5	150	50 °C	3	10	2
				5	15	2
				7	10	2
			100 °C	3	5	--
				5	3	--
				7	2	--
			150 °C	3	--	--
				5	--	--
				7	--	--

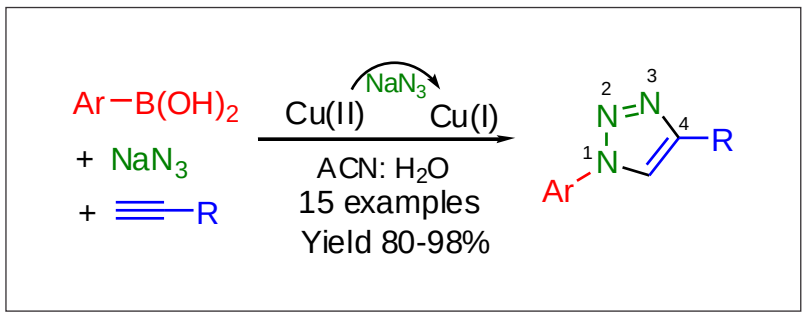
[a]Isolated yields

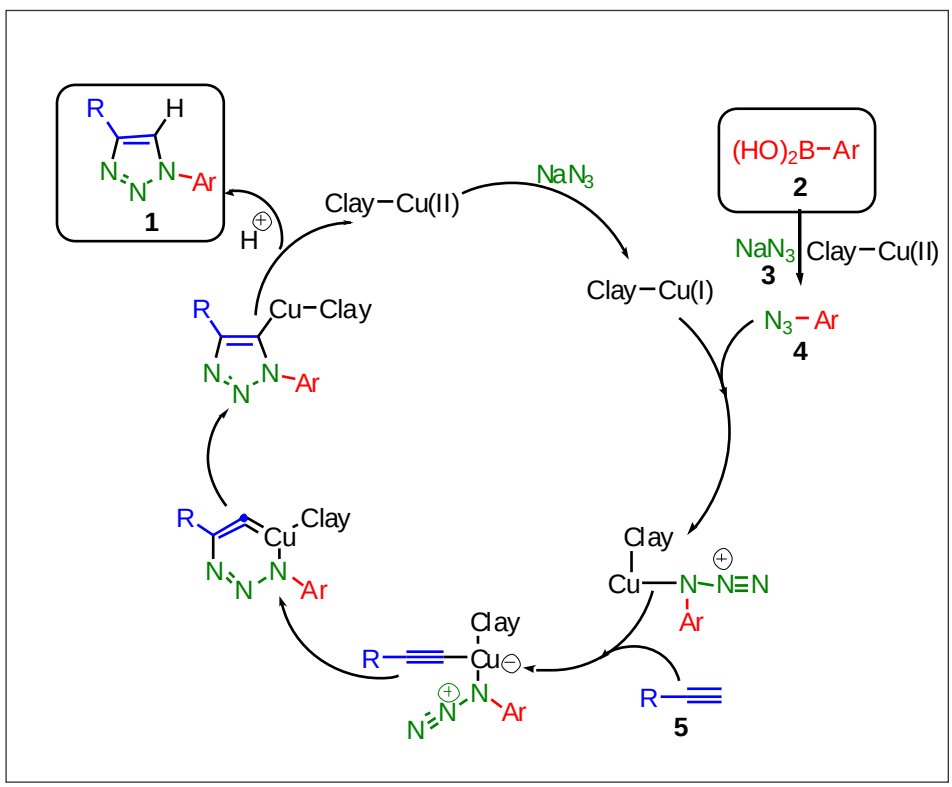
efficient microwave assisted one pot multicomponent method for the synthesis of biologically important purine quinazolinone derivatives. The procedure involves simple protocol and one pot conversion under microwave irradiation which gives the desired purine quinazolinones in good yields. The biological potential of these compounds is being studied.

7.4 Recyclable clay supported Cu (II) catalyzed tandem one-pot synthesis of 1-aryl-1,2,3-triazoles(*Tetrahedron*, **2012**, *68*, 8156-8162).

Shabber Mohammed, Anil K. Padala, Bashir A. Dar, Baldev Singh, B. Sreedhar, Ram A. Vishwakarma and Sandip B. Bharate

Montmorillonite KSF clay supported CuO nanoparticles efficiently catalyzes one-pot aromatic azidonation of aryl boronic acids followed by regioselective azide-alkyne 1,3-dipolar cycloaddition (CuAAC) reaction producing corresponding 1-aryl-1,2,3-triazole derivatives at room temperature in excellent yields without use of any additives. Investigations on mechanism of CuAAC revealed that sodium azide which is used as azidonating reagent in one-pot protocol reduces Cu(II) to click-active Cu(I). The catalytic efficiency of another Cu(II) source $CuSO_4$ in combination with NaN_3 for this one-pot CuAAC protocol, further supported our mechanism. This is the first report for use of Cu(II)/NaN_3 catalytic system for CuAAC protocol. The clay-Cu(II) catalyst being ligand-free, leaching-free, easy to synthesize from inexpensive commercially available precursors, recyclable and environmentally friendly will be highly useful for economical synthesis of 1,4-disubstituted 1,2,3-triazoles.

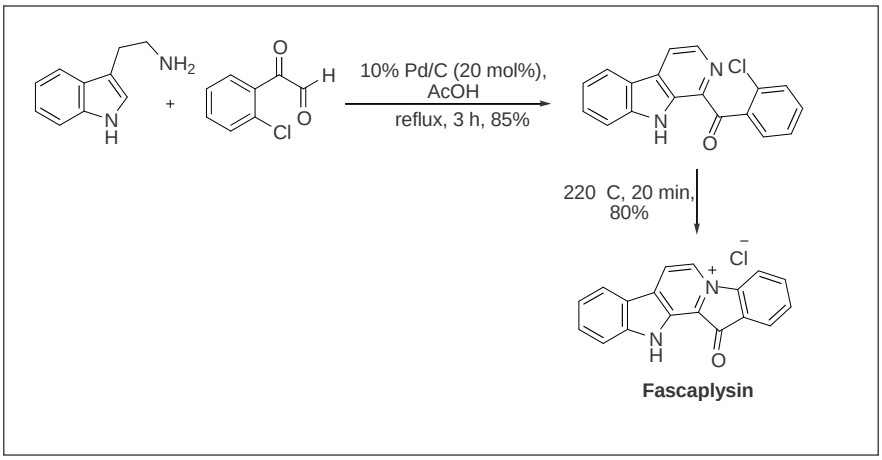




7.5 Total synthesis and anti-cholinesterase activity of marine-derived bis-indole alkaloid fascaplysin

Sandip B. Bharate, Sudhakar Manda, Prashant Joshi,[a] Baljinder Singh and Ram A. Vishwakarma

A short and efficient two-step total synthesis of marine-derived bis-indole alkaloid fascaplysin starting from commercially available tryptamine in 68% overall yield is reported. A key step involved in the present strategy is tandem dehydrative condensation between ortho-halo substituted glyoxal with tryptamine followed by dehydrogenation.



Fascaplysin inhibited acetylcholinesterase (AChE) in non-competitive manner with IC_{50} and *ki* values of 1.49 and 2.28 µM respectively and with 60-fold selectivity for AChE *versus* butyrylcholinesterase.

AChE possesses an active site (cationic anionic site) at which substrates like ACh and ATCh are hydrolyzed and a peripheral anionic site, which is spatially distinct from the active center and where selective inhibitors bind with high affinity and specificity. Hydrolysis of ACh (and ATCh) proceeds through the formation of an acyl-enzyme intermediate, and inhibitors can affect steady-state kinetics by association with the transient acyl-intermediate in addition to the free enzyme and enzyme-substrate complex. In order to understand the mechanism of inhibition for fascaplysin, kinetic studies were carried out. The type of inhibition was elucidated from the analysis of Lineweaver-Burk reciprocal plot . As depicted in figure, lines crossing the x-axis at same point reveal unchanged Km and decreased V_{max} at increasing inhibitor concentrations. This is a typical trend for non-competitive inhibition and indicates that fascaplysin binds with high affinity to a site different from the catalytic one. The inhibition rate constant (*ki*) value was determined from the replot of slopes of reciprocal plot *versus* inhibitor concentrations (Figure b).

Table. In vitro AChE and BuChE inhibitory activity of fascaplysin (**1**)

Inhibitor	**IC_{50} (µM)[a]**		**Selectivity**
	AChE[b]	BuChE[c]	BuChE/AChE
Fascaplysin		90.47 ± 4.07	60.7
Donepezil	0.09 ± 0.04	5.52 ± 1.05	61.3

[a] Values are mean ± SEM of at least three independent measurements.
[b] *Electrophorus electricus* acetylcholinesterase (eeAChE)
[c] Equine serum butyrylcholinesterase (eqBuChE)

The overlay image of the fascaplysin (1) and donepezil docked into the AChE active site gorge is shown in Figure 6. Donepezil (Aricept), a dual-site binding nanomolar inhibitor of AChE, interacts with both catalytic as well as peripheral site residues of the gorge, whereas, fascaplysin (1) spans only in the peripheral and middle portion of the gorge. The overlay image (Figure 6) of fascaplysin (1) and donepezil in the AChE active site gorge indicates that fascaplysin (1) is shorter in length and does not reach to the catalytic site, as indicated by its distance of 6.07 A from the key CAS residue Trp84. Based on docking studies, the lower potency of fascaplysin (1) in comparison to donepezil can be attributed to presence of: (a) lower hydrophobic fitting and ?-? stacking interactions; (b) lesser hydrogen bonding interactions; (c) no interaction with a key catalytic site residue Trp84.

The anti-cholinesterase activity of fascaplysin (1), we discovered herein, can be used as a starting point to discover fascaplysin-based potent anti-cholinesterase compounds which can emerge as potential anti-Alzheimer's agent (s).

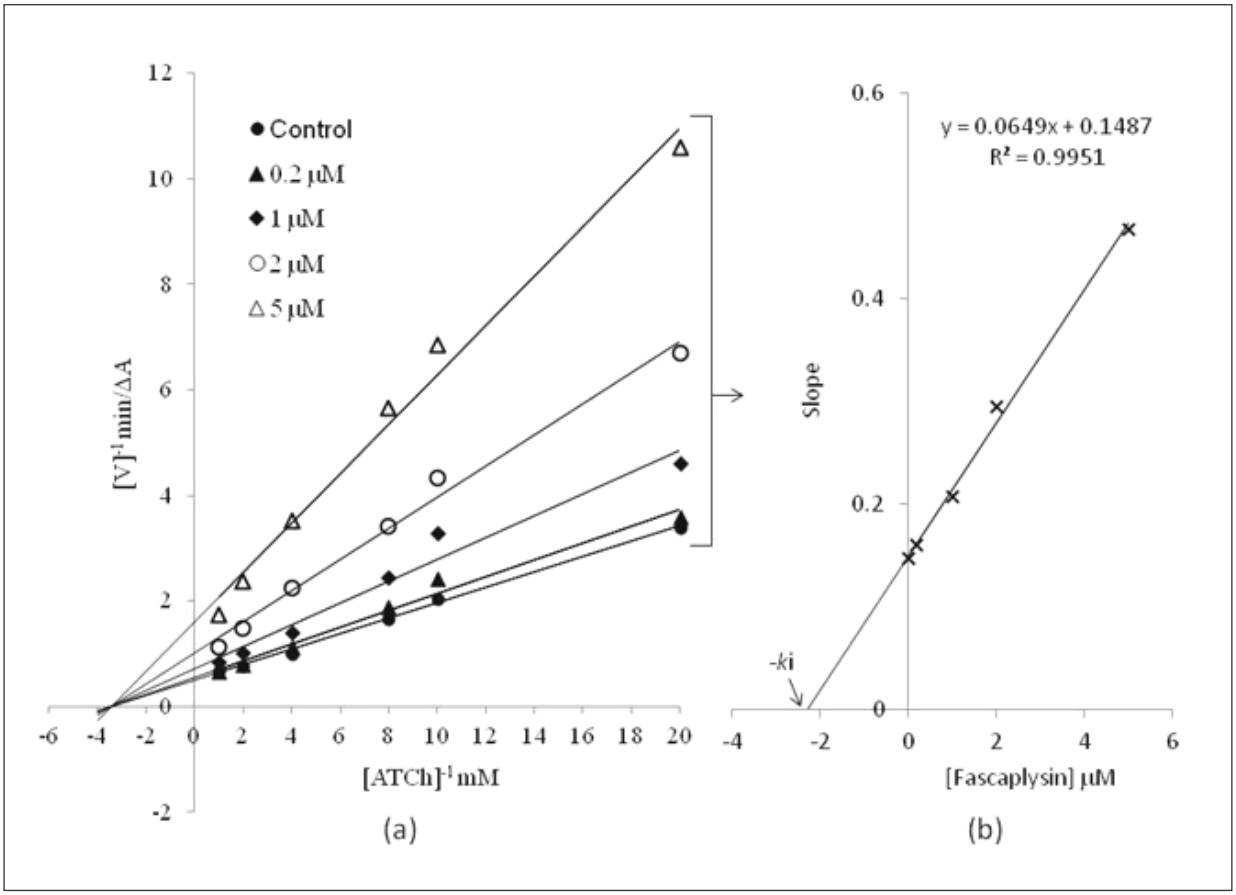


Figure. Steady-state inhibition of AChE hydrolysis of ATCh by fascaplysin (1). (a)Lineweaver-Burk reciprocal plots of initial velocity and substrate concentrations (0.05-1 mM) are presented. (b) Estimation of *k*i for fascaplysin from slope replot*versus* inhibitor concentration.

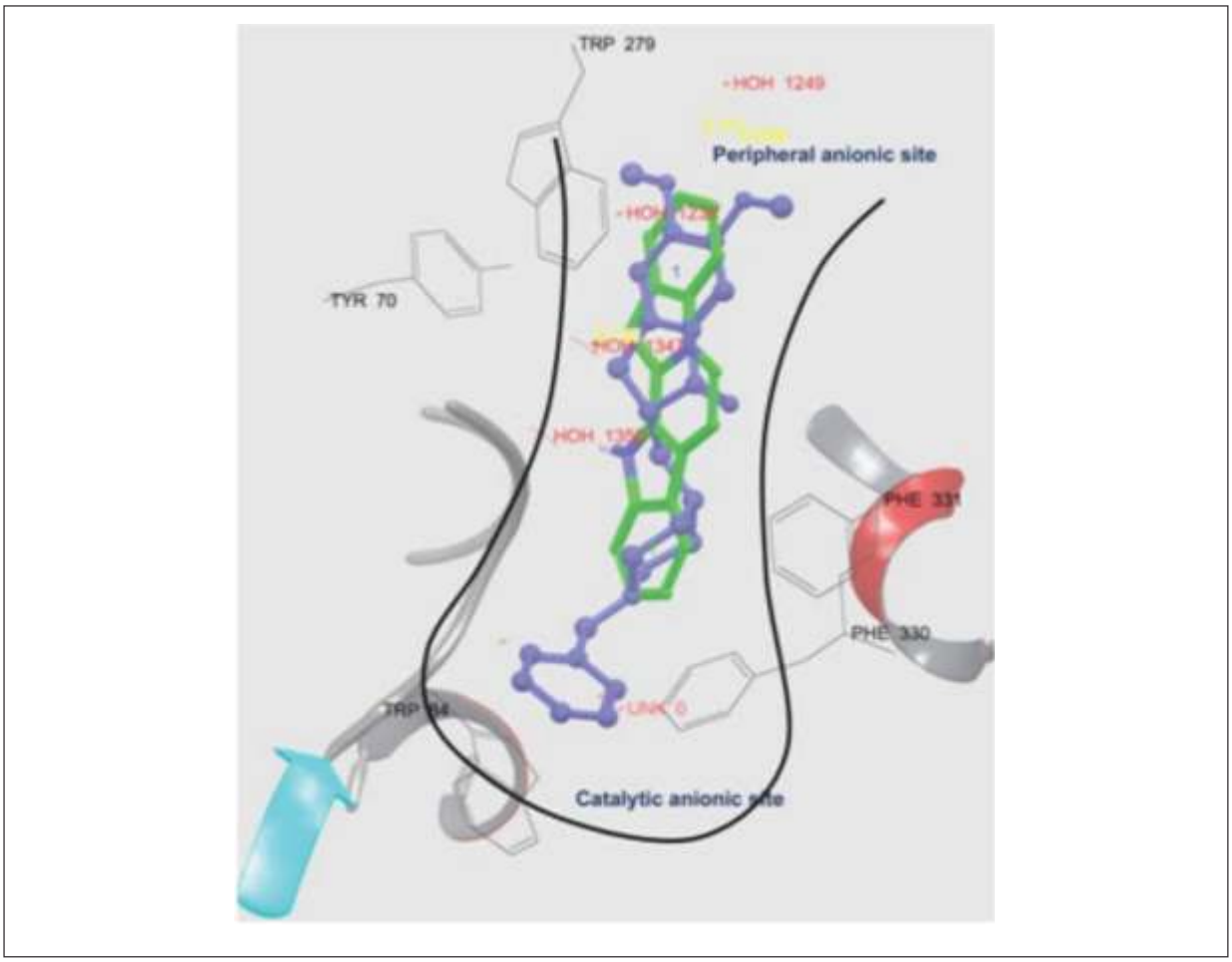
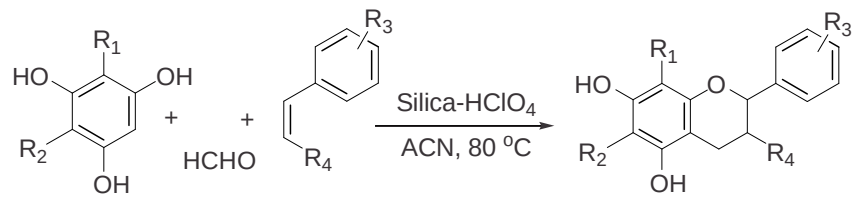


Figure. Overlay image of fascaplysin (**1**) and donepezil in the AChE active site. The green colored ligand is fascaplysin (**1**) and blue colored ligand is donepezil.

7.6 Tandem One-Pot Synthesis of Flavans by Recyclable Silica-HClO4 catalyzed Knoevenagel Condensation and [4+2]-Diels-Alder Cycloaddition

Sandip B. Bharate, Ramesh Mudududdla, Jaideep B. Bharate, Narsaiah Battini, Satyanarayana Battula, Rammohan R. Yadav, Baldev Singh and Ram A. Vishwakarma



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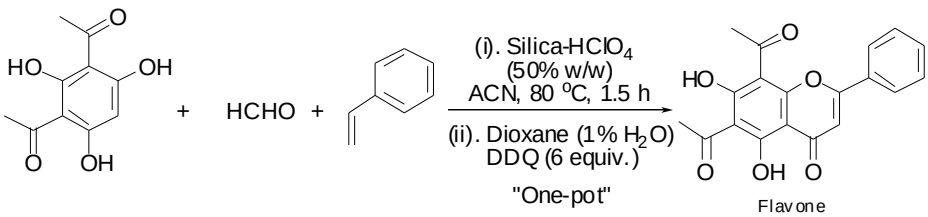
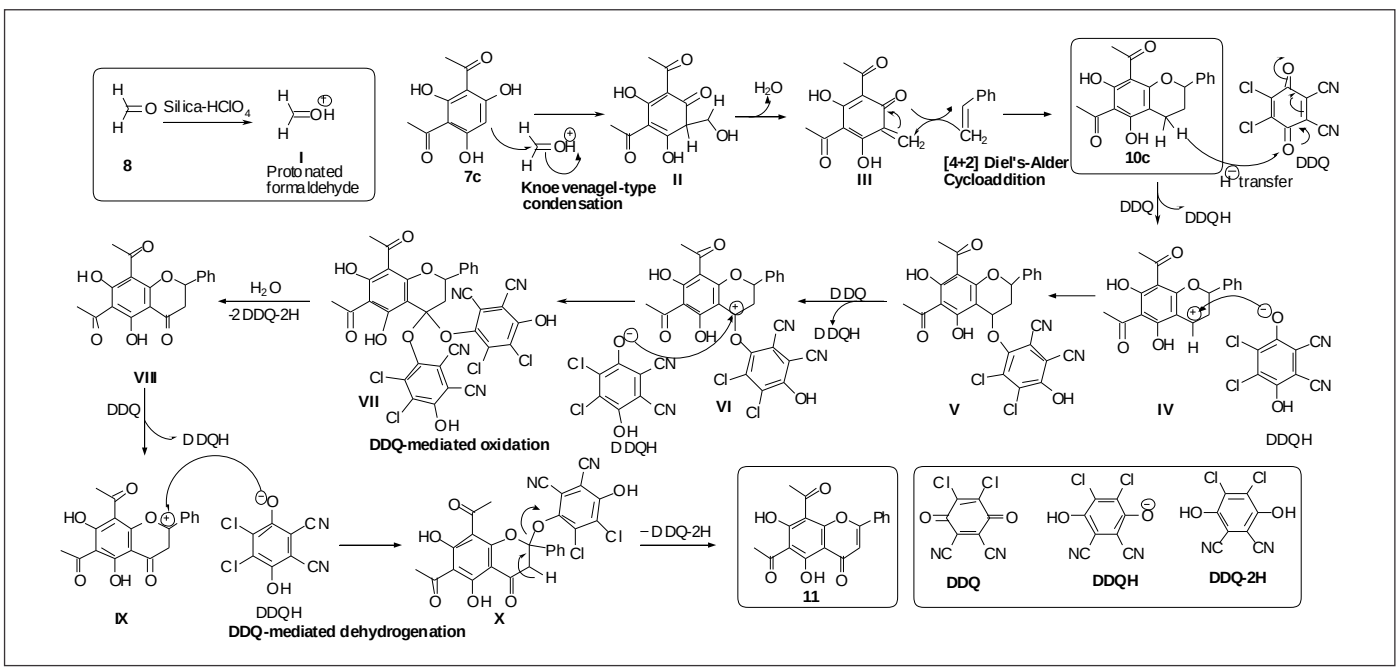
An efficient one-pot multi-component synthesis of flavans using perchloric acid supported on silica as a recyclable heterogeneous catalyst has been described. This is the first report of direct one-step construction of flavan skeleton from phenolic precursor. The method involves Knoevenagel-type [4+2]-Diels-Alder cycloaddition with styrene to yield flavan skeleton.

Further, the utility of this protocol was explored for flavonoid synthesis. An optimized one-pot protocol for flavone synthesis directly from phloroglucinols include, evaporation of ACN after to formation of flavone in 65% yield.

The formation of flavone 11 via one-pot protocol from phoroglucinol precursor 7c involves cascade of 4 reactions namely, Knoevenagel-type condensation, [4+2] Diels-Alder cycloaddition, DDQ-mediated oxidation and DDQ-mediated



condensation leading to in-situ formation of transient *O*-quinone methide which further undergoes flavan formation followed by addition of DDQ and dioxane (1% water) in the same pot, which leads dehydrogenation. The plausible mechanism for this one-pot MCR is depicted below.

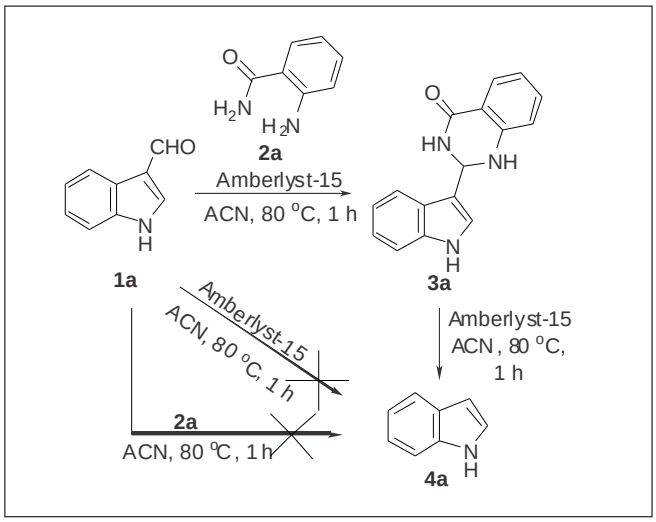


The method provides easy access to wide range of bio-active natural products *viz*. flavonoids, anthocyanins and catechins.

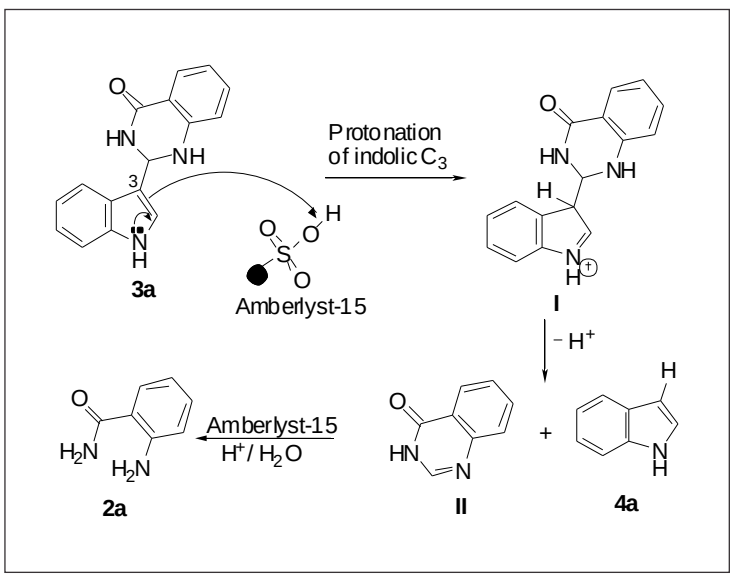
7.7 Deformylation of indole and azaindole-3-carboxaldehydes using anthranilamide and solid acid heterogeneous catalyst via quinazolinone intermediate.

Rammohan R. Yadav, Narsaiah Battini, Ramesh Mudududdla, Jaideep B. Bharate, Nagaraju Muparappu, Sandip B. Bharate and Ram A. Vishwakarma

The deformylation of indole and azaindole-3-carboxaldehyde was achieved in presence of anthranilamide and a solid acid heterogeneous catalyst under reflux conditions in 25-90% yield. The reaction proceeds via quinazolinone intermediate, which undergoes acid catalyzed cleavage to form deformylated product. Both solid acid catalysts (amberlyst-15 and silica-$HClO_4$) are recyclable and can be reused several times without significant loss of activity.



The mechanism of deformylation of indole aldehydes was then predicted. It is evident that, the presence of anthranilamide as well as acidic condition is essential for deformylation of indole 3-carboxaldehydes, and quinazolinone 3a is the key intermediate in this process. Further to unravel the mechanism of quinazolinone (3a) cleavage, firstly the possible role of CN nucleophile (from acetonitrile solvent) in this process was investigated. The reaction of 1e with 2a in presence of silica-$HClO_4$ in other solvents such as dioxane, methanol and THF was performed under optimized reaction conditions. The desired deformylated product 4e was formed in all three solvents in 20, 55 and 35% yield respectively. These results ruled out the role of CN nucleophile in the mechanism. As depicted in above figure, we speculated that under acidic environment, indolic C_3-position gets protonated resulting in formation of iminium ion intermediate I with disrupted aromaticity. Iminium form of indole is highly unstable; thus the intermediate I gets cleaved leading to formation of indole 4a. The expected side product of this cleavage, quinazolinone II was not detected; however we observed formation of anthranilamide (2a) in the reaction mixture. This indicates that quinazolinone II can be further cleaved to anthranilamide 2a under acidic condition as reported earlier. The indole 4a was formed in good yield (~80%), however the mass balance for anthranilamide portion of 3a was not observed, as only 10% yield of 2a was obtained. The probable mechanism for cleavage of quinazolinone intermediate 3a leading to formation of indole 4a and anthranilamide 2a is depicted in below.



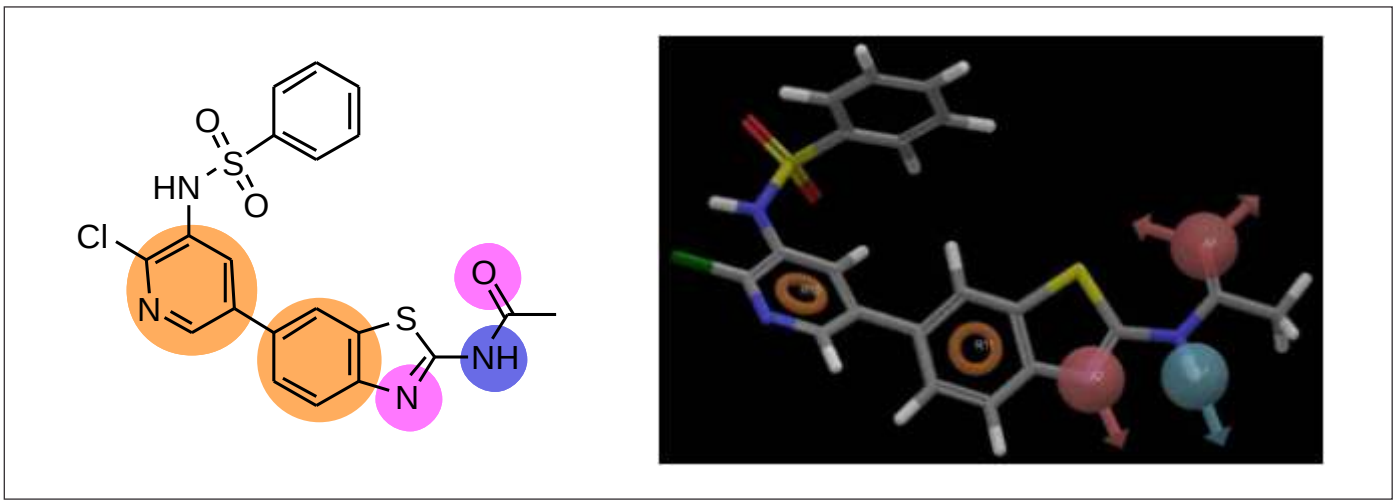
In conclusion, we have established a novel, wide applicability, eco-friendly, mild conditioned deformylation of indole and azaindole-3-carboxaldehydes using reusable heterogeneous catalysts. Conceptually, the work described provides a useful strategy in multi-step synthetic protocols, wherein an aldehyde acts as an activating/directing group and is subsequently removed or excised. When this concept has been used previously the excisable groups were limited to heteroatom-based systems, such as sulfones or nitroalkanes. The deformylation strategy we have described may have additional useful applications in indole/azaindole based chemistry. Furthermore, present paper adds the new use of heterogeneous solid acid catalysts silica-$HClO_4$ and amberlyst-15.

7.8 QSAR and pharmacophore modeling of N-acetyl-2-aminobenzothiazole class of phosphoinositide-3-kinase-inhibitors.

Sandip B. Bharate, Baljinder Singh, Jaideep B. Bharate, Shreyans K. Jain,Samdarshi Meena and Ram A. Vishwakarma

The mTOR-mediated PI3K/AKT/mTOR signal transduction pathway plays key role in a broad spectrum of cancers. In the present article, QSAR and pharmacophore studies were carried out using a series of 61 benzothiazole class of PI3K? inhibitors to characterize molecular features and structural requirements crucial for biological interaction. QSAR study performed using TSAR 3.3 by multiple regression analysis and partial least square methods identified inertia moment-1-size, kier chiv5 (path) index and number of H-bond donors as important descriptors responsible for PI3K? inhibitory activity. Further analysis of pharmacophore model using Phase module of Schrodinger revealed that two hydrogen bond acceptors (A), one hydrogen bond donor (D) and two hydrophobic aromatic rings (R) as crucial molecular features that predict binding affinity for high affinity ligands to the PI3K? enzyme. The best Phase hypothesis (AADRR.19) superimposed on the one of the most active compound is shown below. orange: hydrophobic aromatic; pink: H-bond acceptor; blue: H-bond donor.

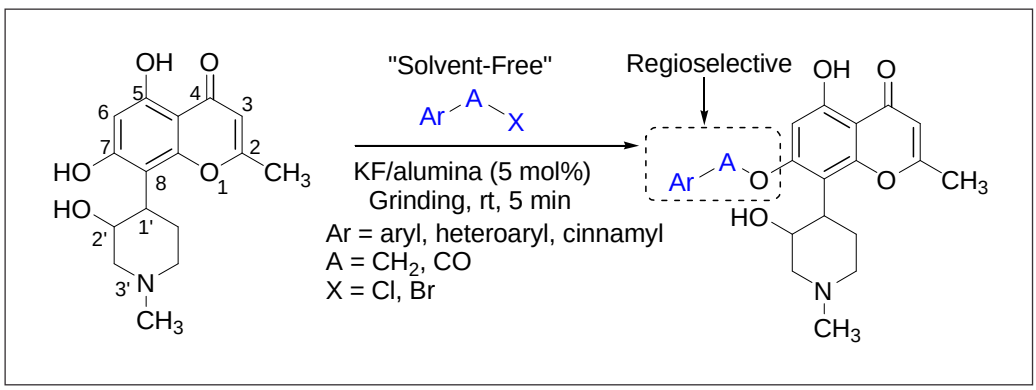
These observations provides important insights to the key structural requirements of these molecules for potent PI3K? inhibition. Excellent statistical results of developed models strongly suggest that these models are reasonable for the prediction of the activity of new inhibitors and in future drug design.

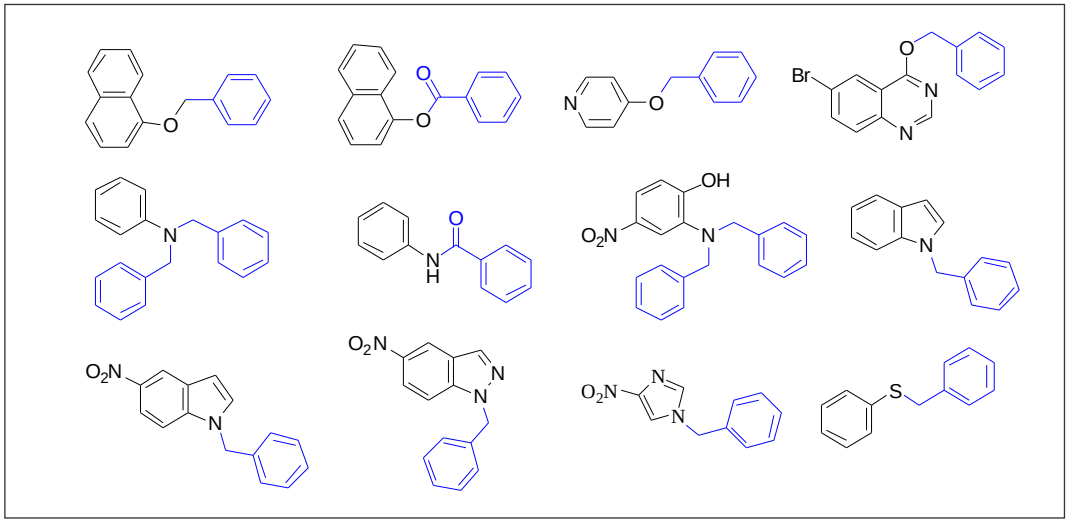


7.9 KF/alumina catalyzed regioselective benzylation and benzoylation using solvent-free grind-stone chemistry

Shreyans K. Jain, Samdarshi Meena, Baljinder Singh, Jaideep B. Bharate, Prashant Joshi, Varun P. Singh, Ram A. Vishwakarma and Sandip B. Bharate

Potassium fluoride-impregnated on alumina catalyzes solvent-free regioselective *O*-benzylation, benzoylation and cinnamylation of phenols. Reaction proceeds simply by triturating together equivalent amounts of phenol and corresponding halide in presence of 5 mol% of KF/alumina for 5-20 min in mortor pestle, without need to any additive such as phase-transfer catalyst or solvent. Key features of the protocol include its efficiency also for solid-solid





precursors and regioselectivity for phenolic hydroxyls *versus* alcoholic hydroxyls. Utility of the protocol for *N*- and *S*-benzylation has also been explored. Representative examples are shown below.

Products were obtained in excellent yields and catalyst can be easily recycled several times without significant loss of activity. The catalyst used is easy to prepare from inexpensive chemicals that are commonly found in organic chemistry laboratories, thus this procedure can be easily adopted in process chemistry research. Apart from its green chemistry aspects, this method is convenient and time-saving in comparison to routine reflux reactions. Routine methods of O- alkylation and acylation such as NaH/DMF, K_2CO_3/acetone were found to be inefficient for O-benzylation/ benzoylation of rohitukine. However, KF-alumina catalyzed grind-stone chemistry led to completion of reaction within 5 min producing solely mono-benzylated/ mono-benzoylated products in excellent yields. Thus, results obtained clearly indicate KF/alumina catalyzed solvent-free regioselective O-benzylation and O-benzoylation of rohitukine. Results obtained herein, further guaranties utility of this protocol for benzylation of structurally diverse phenols over alcohols.

8. FERMENTATION TECHNOLOGY DIVISION

8.1 Immobilization of enantioselective lipase on soluble supports for kinetic resolution of drug intermediates

Asha Chaubey, S.C. Taneja and R. Parshad, Sarika Deokar, C.R. Rajan and S. Ponrathnam.

An efficient enantioselective lipase i.e. *Arthrobacter* sp. Lipase from IIIM repository (ABL, MTCC 5125) has been identified as an efficient enzyme for kinetic resolution of various drug intermediates with high enantioselectivity. ABL immobilization on soluble linear supports was carried out on copolymers of N-vinyl pyrrolidone-allylglycidyl ether (ANP type) and N-vinyl pyrrolidone-glycidyl methacrylate (GNP type) by covalent binding through the epoxy groups available on the supports. These polymers have soluble-insoluble characteristics at different pH range. Such polymers are advantageous over insoluble polymers as the soluble form of polymer at neutral pH provides better accessibility of immobilized enzyme to the insoluble substrates and can be precipitated at low pH (pH 2-3) for reuse. Kinetic resolution of racemic acyl derivatives of chiral auxiliaries and drug intermediates viz. phenyl ethanol, amino alcohol and fluoxetine intermediate have shown a significant enhancement in enantioselectivity up to 99% ee.

Immobilization of ABL on soluble Polymers :

Copolymers obtained from various combination of N-vinyl pyrrolidone(NVP) and allylglycidyl ether(AGE)/glycidyl methacrylate(GMA) used in the present study have reactive epoxy groups that can directly bind to the free amino groups of enzyme. Among all the polymers chosen for present study, ANP1 and GNP1 polymers were found to have more binding capacity (100-110 mg/g support) due to presence of higher epoxy content as compared to ANP2, GNP2 with protein binding of 90-95mg/g support. However, the protein binding was not found proportional to the available epoxy content. Similarly the activities of immobilized ABL were higher in ANP1(700U/g) and GNP1(600U/g) polymers as compared to ANP2 (600U/g) and GNP2(500U/g) polymers. On re-precipitation, there was no significant change in immobilized enzyme activity.

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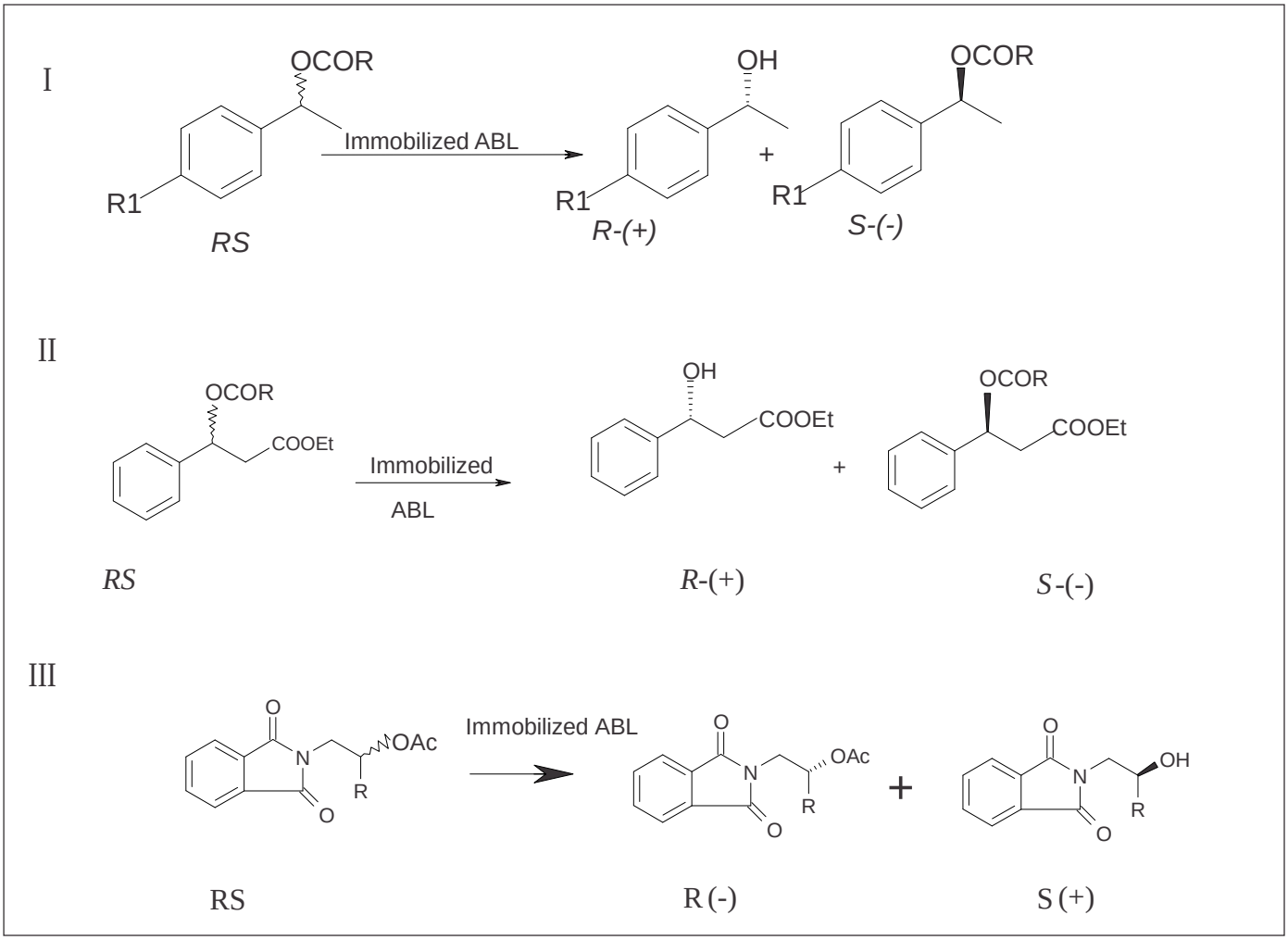
Kinetic resolution of drug intermediates:

All the immobilized ABL soluble supports were tested for their kinetic resolution efficacies during hydrolysis of chiral auxiliary 1-phenyl ethanol acylates. The results provided hydrolysed product (R) isomer at 38-42% conversion with 99% enantioselectivity after 24h as compared to wet cell free enzyme providing only upto 94-95% enantioselectivity. Resolution of racemic fluoxetine intermediate acylates also presented 99% ee at 35-40% conversion after 24h reaction as compared to 93-94%ee in case of cell free enzyme. These results are similar to those obtained with ABL immobilized on insoluble polymers and both types of polymers provided hydrolysed product of liquid substrates with 99% enantioselectivity.

Hydrolytic resolution of -aminoalcohols using ABL cell free enzyme extract provided only upto 70% enantioselectivity (ee). The rate of reaction and enantioselectivity

Table I: Immobilized enzyme activity and protein on different soluble polymers

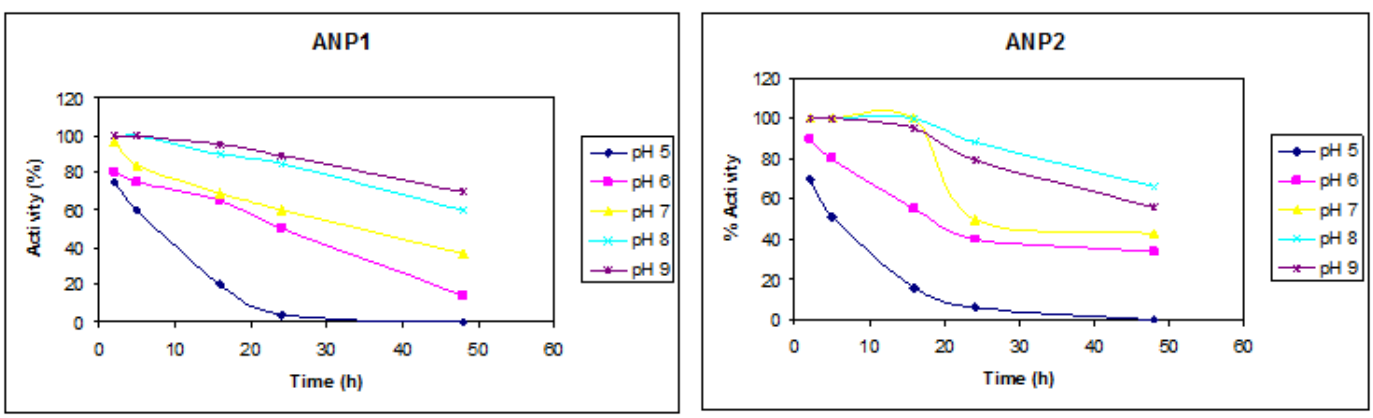
Polymer code	Epoxy content (mol/g)	Immobilized enzyme activity (U/g support)	Protein binding (mg/g support)
ANP1	0.00072	700	100
ANP2	0.000449	600	95
GNP1	0.0008753	600	110
GNP2	0.0004436	500	90

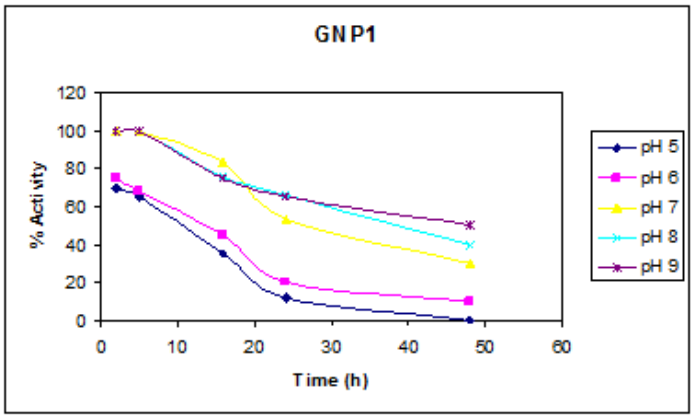


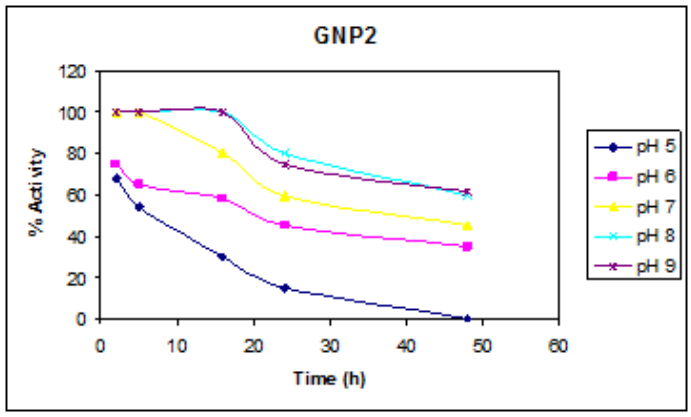
significantly increased on using immobilized ABL on soluble supports providing maximum 99% enantioselectivity at 43% conversion in 24h. On further proceeding of the reaction, enantioselectivity decreased to 97% at 48% conversion using ANP type support. Resolution of Substrate III using ABL enzyme immobilized on soluble polymers improved enantioselectivity up to 99% at 20-22% conversion and 91-92% ee at 35-49% conversion.

Reversibly soluble-insoluble immobilized ABL preparations from copolymers of N-vinyl pyrrolidone-allylglycidyl ether (ANP type) and N-vinyl pyrrolidone-glycidyl methacrylate (GNP type) showed enhanced stability of the enzyme as compared to free enzyme and presented a facile method for the preparation of enantiopure 1-phenyl ethanol, fluoxetine intermediate and -amino alcohols (99%ee) with

Fig2.: pH stability of immobilized ABL on ANP and GNP type water soluble polymers





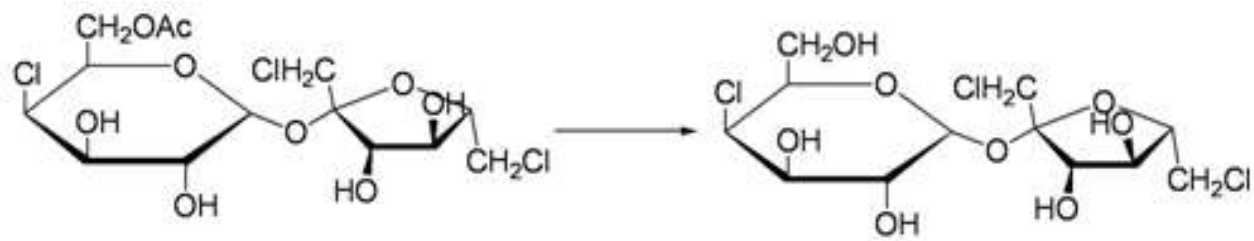


enhanced stability.The soluble immobilized enzyme preparations presented better reaction rates as well as enantioselectivity (99% ee) for resolution of insoluble substrates, which was not possible using immobilized enzyme on insoluble supports.

8.2 Bioconversion of sucralose-6-acetate to sucralose using immobilized microbial cells .

Asha Chaubey, C. Raina, S.C. Taneja and R. Parshad

Biotransformation process for the production of Sucralose, an artificial sweetener has been reported using whole cells and immobilized whole cells of *Arthrobacter* sp. and *Bacillus subtilis* strains isolated at IIIM, Jammu, India. Immobilized whole cells packed bed reactor has shown much superior biotransformation process using sucralose-6-acetate to sucralose in aqueous system where purification of the final product is not required. The reactor was used more than three cycles much cheaper and less time consuming.



Sucralose-6-acetate Sucralose

Sucralose, trichlorogalactosucrose or 4,1',6'-trichlorogalactosucrose is known as an artificial sweetener having 600 times sweetening ability than sucrose. It is used in place of sugar to eliminate or reduce calories in a wide variety of products, including beverages, baked goods, desserts, dairy products, canned fruits, syrups. Sucralose is chemically known as 1,6-dichloro-1,6-dideoxy-beta-D-fructofuranosyl-4-chloro-4-deoxy-alpha-D-galactopyranoside and has been derived from sucrose by replacing the hydroxyl groups in the 4, 1', and 6' positions with chlorine.

First report on sucralose appeared in 1987 by Queens Elizabeth College in London and Tate & Lyle, a private company where, chemical modification of sucrose by selective replacement of three hydroxy groups with chlorine atoms was reported. Canada was the first country to approve use of sucralose in foods in 1991 followed by FDA the United States in 1998. It is now being used in at least 28 countries as sweetening agent. Sucralose is being sold under the brand name Splenda by McNeil Specialty Products Company, New Brunswick, New Jersey. About 120 products using sucralose as a sweetener are in the U.S. market and other countries are also using surcalsoe as sweetener in food products.

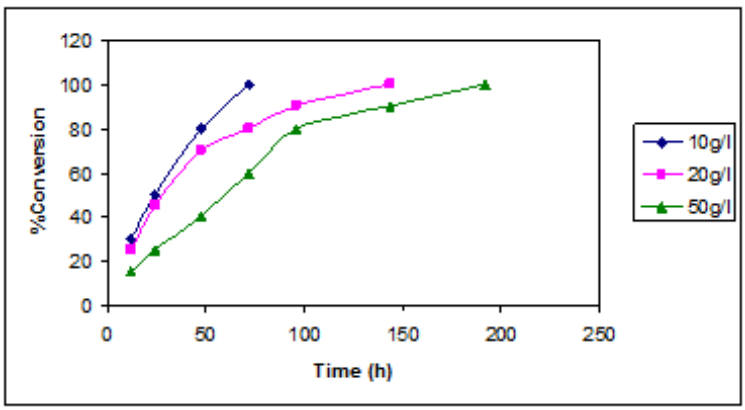
Sucralose is generally synthesized by chemical methods involving five-step process by selective substitution of three chlorine atoms for three hydroxyl groups in the sucrose molecule. Such methods involve several purification steps to get pure sucralose.

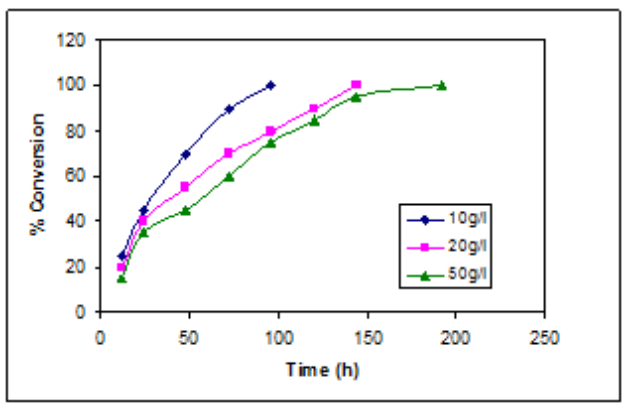
We have hydrolysed sucralose-6-acetate to sucralose enzymatically in a single step using acyl hydrolase enzyme from IIIM Jammu, India microbial isolates; *Arthrobacter* sp.

and *Bacillus subtilis*. The bioconversion of sucralose-6-ester has been carried out in aqueous system without pH adjustment or presence of buffering salts using free enzyme/cells/immobilized enzyme/immobilized cells; therefore the final product does not require any purification.

Substrate at 10-20g/l was completely hydrolysed by entrapped *Bacillus subtilis* cells in 72-96h. On further increasing the substrate concentration (50g/l), rate of reaction was much slower resulting in 100% biotransformation in 7 days(168h). Entrapped microbial cells from *Arthrobacter* sp. showed slower reaction rates with production of pure product in 9 days(216h). The reaction was terminated by centrifugation and concentrated under vacuum for crystallization of product. The product purity was tested by HPLC, optical rotation and NMR.

Biotransformation of sucralose-6-acetate to sucralose in immobilized whole cells reactor of *Bacillus subtilis* (a) and *Arthrobacter sp. lipase* (b)



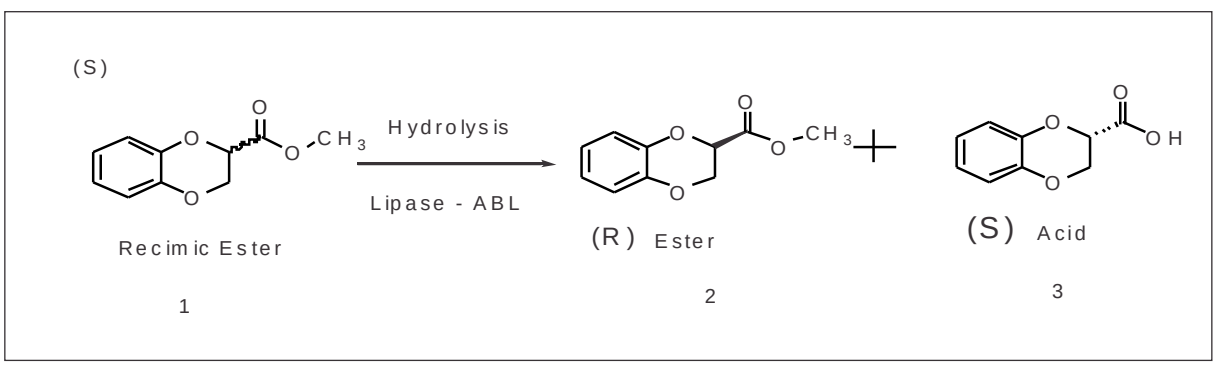


8. 3 Kinetic resolution of (±)-1,4-benzodioxan-2-carboxylic acid:

Asha Chaubey, A. Rouf, S.C. Taneja and R. Parshad

Doxazosin mesylate belongs to quinazoline group of drugs; it is used in the treatment of hypertension and benign prostatic hyperplasia.Compounds containing enantiomerically pure 1,4-benzodioxin and 1,4-benzodioxan structures have attracted considerable interest in recent years, mainly due to their significant contribution in medicinal chemistry and are found in a variety of biologically active natural products.Recently, it has been found that both the enantiomers of (±)-1,4-benzodioxan-2-carboxylic acid, used as an intermediate for the synthesis of doxazosin mesylate, have different biological activities

In order to assess the effect of immobilized enzyme (ABL), we performed hydrolytic reactions with whole cells/entrapped isolated enzyme and entrapped whole cells.



ABL whole cells and isolated enzyme were entrapped in sol-gel supports and dry powder containing entrapped enzyme/cells was directly used for resolution studies. It was found that selectivity as well as conversion was increased by immobilizing the whole cells and isolated enzyme. For reuse, immobilized whole cells/enzyme was separated from the reaction mixture by centrifugation and washed well with solvent/sodium bicarbonate/ distilled water to remove any traces of substrate/product. The immobilized enzyme was dried again before proceeding for next reaction cycle.

Using the immobilized ABL, both the enantiomers of 1,4-benzodioxan-2-carboxylicacid (key intermediate in the synthesis of doxazosin mesylate), were obtained with good yields and excellent enantioselectivity (ee >99%, E >400).

9. ENZYMOLOGY AND BIOTRANSFORMATIONS

9.1 Evaluation of the Catalase promoter for expressing the alkaline xylanase gene (alx)in Aspergillus niger

R. Sharma, N. Govindappa, P. S. Shastry and M katoch

Aspergillus niger represents a promising host for the expression of recombinant proteins but only a few expression systems are available for this organism. In this study, the inducible catalase promoter (P*cat*R) from *A. niger* was characterized. For this, constructs were developed and checked for the expression of the alkaline xylanase genetranscriptionally fused underthe *cat R* promoter.

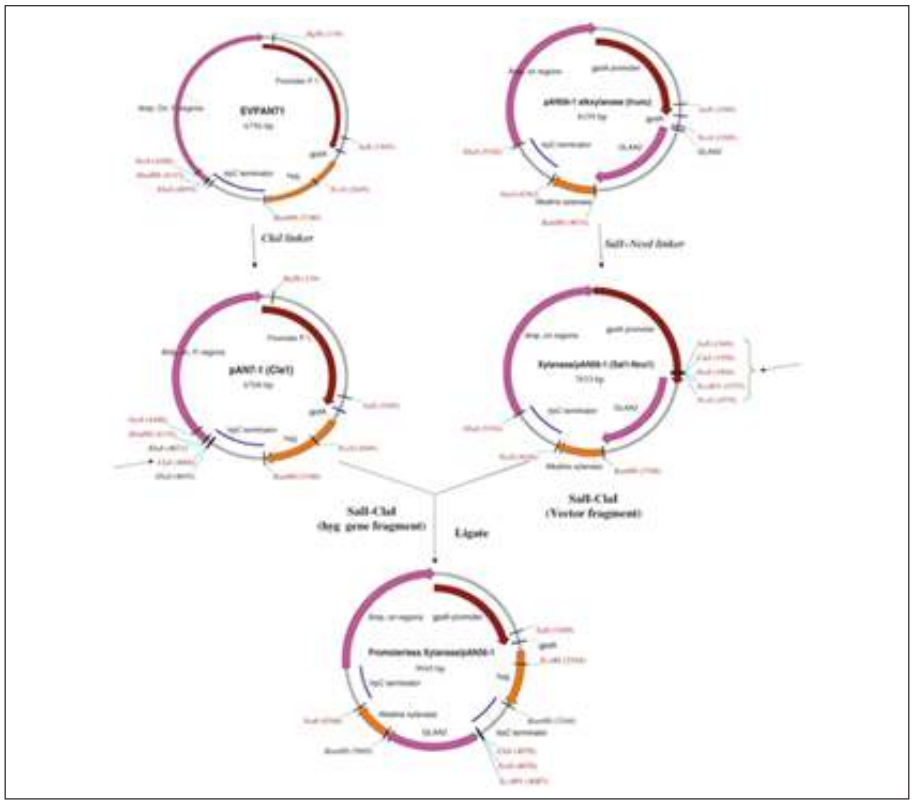


Figure 1.Construction of promoter-less xylanse/pAN56-1 vector.hyg, hygromycin resistance marker; AlX, alkaline xylanase gene; ori, *Escherichia coli* origin of replication.

Figure 2.Sequence of the *catR* promoter of the *Aspergillus niger* highlighting the TATA-, CAAT- motifs in gray, heat shock transcription factor motifs in bold and italics, and cre motifs in bold, highlighted and underlined with double line. Nucleotides are numbered from the putative translation initiation codon (ATG) indicated as - 1 above.

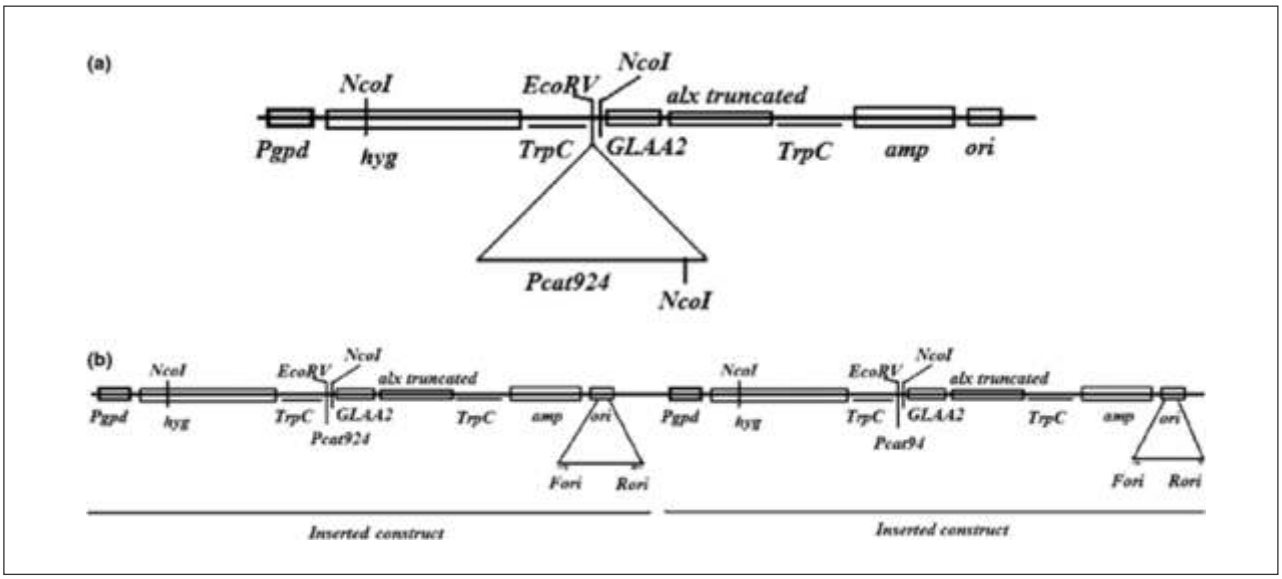


Figure 3.(a) Restriction digestion of promoter-less xylanase/pAN56-1 vector. (b) Integration of construct in *Aspergillus niger* genome and Scheme to confirm the integration of construct in *A. niger* genome by using the *Escherichia coli ori* primers

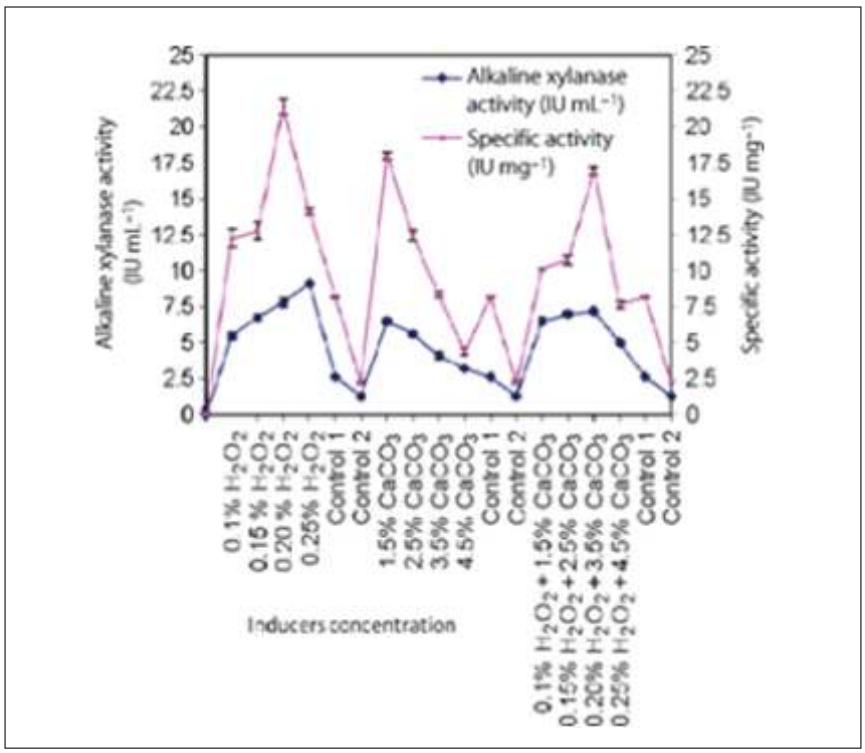


Figure 4.Effect of different inducers and their concentrations on AlX activity of transformant K6. The AlX activity of the K6 transformant grown with inducers was compared with K6 transformant grown under non-induction conditions (control 1) and transformant harboring promoter-less construct (control 2).

Two versions of the catalase (*catR)* promotersequence from *A. niger*($P_{cat300,}$ P_{cat924}) were isolated and tested for their ability to drive expression of the alkaline xylanase (*alx)* gene. P_{cat924} showed better efficiency (more than ten fold increase in AlX activity compared toP_{cat300}) under the optimized culture conditions.Induction of the *catR* promoter with 0.20 % $H_2O_2$and 1.5% $CaCO_3$in the culture medium, further increased expression of AlXto 2.61 and 2.20 fold respectively clarifying its inducible nature. Specific induction or repression of the *catR* promoter provides the possibility for utilization of this promoter in heterologous protein production.In this study, we sought to exploit *catR* promoter to produce recombinant protein. For this purpose, two promoters of different lengths P_{cat300} and P_{cat924} were amplified and cloned in promoterless xylanase/pAN56-1 vector. Both type of transformants harboring $P_{cat(300)}$xylanase/ pAN56-1 and$P_{cat924bp}$xylanase/ pAN56-1 were evaluated for driving the expression of *alx* gene. Expression of AlX in all transformants suggested that $P_{cat(300)}$ contained the sequences required to

initiate the start of transcription. Different AlX activity was found in different transformants (A1-A10 and K1-K10) which might be attributed to varying copy number or varying position in the genome of the host at which integration took place as also reported by Verdoes et al., (1993).

To evaluate the effect of seed media on the AlX expression of transformants, two seed mediums viz Sabauraud's media and wheat flour media were tried. AlX expression was found highest in transformants grown in Sabauraud's media (41.91-91.4 U/mg) in comparison to wheat flour media (5.61-20.72 U/mg) which might be because of better growth of transformants inSabauraud's mediain comparison to wheat flour media. Wheat bran is considered as one of the most popular components of complex media for xylanase production (Deschamps & Huet 1985; Hoq et al., 1994; Sapereira et al., 2002). Many authors reported the advantages of using wheat bran as a substrate for xylanase production, therefore for functional characterization; wet wheat bran was used as production medium.

In Sabauraud's media,transformants A1-A10 showed AlX activity in the range of 46.66 - 80.74 U/mg which depicted 3.21 fold increase inAlX activity. This might be attributed to TATA box present at -59 position in $P_{cat300.}$ The TATA box was the first core promoter element identified in eukaryoticprotein-coding genes (Breathnach & Chambon 1981). In Sabauraud's media, transformants K1-K10 showed AlX activity in the range of 41.91-91.4 U/mg which depicted 3.64-fold increase in AlX activity which might be attributed to two TATAA boxes at position -59 and -359 and two CCAAT motifs lying at -355 and -590 position.As reported by Bucher, (1990), in filamentous fungi and higher eukaryotes, the CCAAT motif is as an essential and functional element for high-level expression of a large number of genes.The region from -59 to -590 contains the two TATAA and two CCAAT box and thus was involved in strong expression. As also suggested by Liu et al., (2003) that multiple copies of CCAAT motifs improved the heterologous protein production in *A. niger*. Results discussed here indicating that there was no significant increase in specific activity in K transformants inspite of two CCAAT and two TATAA boxes, which might be because of three cre1 binding sites (5'-SYGGRG-3') present at -98, -613 and -900, responsible for repression by glucose.

In wheat flour media,transformants A1-A10 showed AlX activity in the range of 5.75 – 7.67 U/mg which depicted 3.95 fold increase inAlX activity whereas transformants K1-K10 showed AlX activity in the range of 5.85-20.72 U/mg which depicted 10.3 fold increase in AlX activity which might be attributed to two TATAA boxes, two CCAAT motifs and absence of repression created by binding of glucose with three cre1 binding sites (5'-SYGGRG-3') because of absence of glucose in wheat flour medium. Similarly, Roth et al (2007) using the P*suc*1 promoter observed seven fold increased GFP florescence in recombinant *A. niger*strain.

High expression levels and induction of the *A. niger cat* encoding gene, *catR* by $CaCO_3$ and H_2O_2 have been reported by Liu et al., (1998a & b). The induction of *cat* synthesis by $CaCO_3$ was thought to be either due to the high calcium ion concentration of an insoluble salt which acts as a solid support for mycelial growth or due to resistance against pH change caused by $CaCO_3$. It is also well known that heat shock and hydrogen peroxide induces catalase gene expression in Aspergilli (Abrashev et al., 2005; Hisada et al., 2005), and that each catalase gene promoter has regulatory element for stress response. The AGAAN motifs are consensus DNA binding sites of the heat shock transcription factor (HSF) of *A. oryzae*as reported bylshida et al., (2004).The HSF positively regulates the stress response and the *catR* is involved in defense against oxidative stress in submerged culture, it is anticipated that the AGAAN motifs are involved in the positive regulation of *catR* promoter. The P_{cat924} contained 9 AGAAN sequences which consists of four AGAAN at -701, -692, -555, -498bp in the sense strand and five AGAAN (reverse compliment; NTTCT) at -616, -579, -522, -298, -122 bp in the antisense strand.

Under frequently used P*gla*A of *A. niger*,glucoamylase expression was reported to be 7.5 fold using glucose as inducer vs xylose (Ganzlin and Rinas, 2008). The catR promoter also showed 6.66 fold increase in AlX activity while growing in medium containing maida vs glucose suggesting that the catR promoter is equivalently efficient as P*gla*A of *A. niger*.

Result demonstrated that P_{cat924} showed better efficiency under the given growth conditions.This is the first report describing the identification of the regulatory element of *catR* gene in *A. niger.*Clarifying the specific induction or repression of the *catR* promoter provides the possibility for utilization of this promoter in heterologous protein production industry.

Table 1 AlX activity of transformants in Sabauraud's medium followed by wheat bran

Transformants	**Specific Activity (IU mg^{-1})**	**Fold increase with respect to P_0xylanase/pAN56-1**
P_{cat300}xylanase/pAN56-1#A1	54.28±0.25	2.16
P_{cat300}xylanase/pAN56-1#A2	51.83±0.35	2.06
P_{cat300}xylanase/pAN56-1#A3	67.85±0.38	2.70
P_{cat300}xylanase/pAN56-1#A4	49.03±0.18	1.95
P_{cat300}xylanase/pAN56-1#A5	46.66±0.16	1.86
P_{cat300}xylanase/pAN56-1#A6	64.61±0.25	2.57
P_{cat300}xylanase/pAN56-1#A7	54.31±0.27	2.16
P_{cat300}xylanase/pAN56-1#A8	80.74±0.36	**3.21**
P_{cat300}xylanase/pAN56-1#A9	74.05±0.38	2.95
P_{cat300}xylanase/pAN56-1#A10	75.86±0.23	3.02
P_{cat924}xylanase/pAN56-1#K1	54.21±0.45	2.16
P_{cat924}xylanase/pAN56-1#K2	41.91±0.55	1.67
P_{cat924}xylanase/pAN56-1#K3	46±0.52	1.83
P_{cat924}xylanase/ pAN56-1#K4	52.25±0.38	2.08
P_{cat924}xylanase/pAN56-1#K5	91.4±0.48	**3.64**
P_{cat924}xylanase/pAN56-1#K6	48.33±0.28	1.92
P_{cat924}xylanase/pAN56-1#K7	51.84±0.37	2.06
P_{cat924}xylanase/pAN56-1#K8	45.53±0.54	1.81
P_{cat924}xylanase/pAN56-1#K9	62.59±0.46	2.49
P_{cat924}xylanase/pAN56-1#K10	53.2±0.50	2.12
P_0xylanase/pAN56-1	25.08±0.22	

Table 2 AlX activity of transformants in Wheat flour medium followed by wheat bran

Transformants	**Specific Activity (IU mg^{-1})**	**Fold increase with respect to P_0xylanase/pAN56-1**
P_{cat300}xylanase/pAN56-1#A1	5.75±0.15	2.85
P_{cat300}xylanase/pAN56-1#A2	7.32±0.04	3.63
P_{cat300}xylanase/pAN56-1#A3	6.92±0.28	3.43
P_{cat300}xylanase/pAN56-1#A4	7.17±0.06	3.56
P_{cat300}xylanase/pAN56-1#A5	5.61±0.10	2.78
P_{cat300}xylanase/pAN56-1#A6	7.34±0.05	3.64
P_{cat300}xylanase/pAN56-1#A7	6.81±0.09	3.38
P_{cat300}xylanase/pAN56-1#A8	7.94±0.34	**3.95**
P_{cat300}xylanase/pAN56-1#A9	6.22±0.15	3.09
P_{cat300}xylanase/pAN56-1#A10	7.67±0.035	3.80
P_{cat924}xylanase/pAN56-1#K1	6.4±0.34	3.18
P_{cat924}xylanase/pAN56-1#K2	5.85 ±0.20	2.91
P_{cat924}xylanase/pAN56-1#K3	8.89±0.25	4.42
P_{cat924}xylanase/ pAN56-1#K4	6.18±0.35	3.07
P_{cat924}xylanase/pAN56-1#K5	20.72±0.40	**10.3**
P_{cat924}xylanase/pAN56-1#K6	8.57±0.35	4.26
P_{cat924}xylanase/pAN56-1#K7	11.06±0.25	5.5
P_{cat924}xylanase/pAN56-1#K8	10.7±0.23	5.32
P_{cat924}xylanase/pAN56-1#K9	9.83±0.34	4.89
P_{cat924}xylanase/pAN56-1#K10	13.04±0.36	6.48
P_0xylanase/pAN56-1	2.01±0.120	

10. MICROBIOLOGY

10.1 Purification and characterization of a cold active alkaline protease from *Stenotrophomonas* sp., isolated from Kashmir, India

Iram Saba, Parvaiz H. Qazi, Shabir A. Rather, Refaz A. Dar, Qurrat A. Qadri, Nasier Ahmad, Sarojini Johri, Subash C. Taneja and Sami Shawl.

A Psychrotolerant alkaline protease producing bacterium IIIM-ST045 was isolated from a soil sample collected from the Thajiwas glacier of Kashmir, India and identified as *Stenotrophomonas* sp. on the basis of its biochemical properties and 16S ribosomal gene sequencing. The strain could grow well within a temperature range of 4–37°C however, showed optimum growth at 15°C. The strain was found to over-produce proteases when it was grown in media containing lactose as carbon source (157.50 U mg^{-1}). The maximum specific enzyme activity (398 U mg^{-1}) was obtained using soya oil as nitrogen source, however, the inorganic nitrogen sources urea, ammonium chloride and ammonium sulphate showed the lowest production of 38.9, 62.2 and 57.9 U mg^{-1}. The enzyme was purified to 18.45 folds and the molecular weight of the partially purified protease was estimated to be ~55 kDa by SDS-PAGE analysis. The protease activity increased as the increase in enzyme concentration while as the optimum enzyme activity was found when casein (1% w/v) was used as substrate. The enzyme was highly active over a wide range of pH from 6.5 to 12.0 showing optimum activity at pH 10.0. The optimum temperature for the enzyme was 15°C. Proteolytic activity reduced gradually with higher temperatures with a decrease of 56% at 40°C. The purified enzyme was checked for the removal of protein containing tea stains using a silk cloth within a temperature range of 10–60°C. The best washing efficiency results obtained at low temperatures indicate that the enzyme may be used for cold washing purposes of delicate fabrics that otherwise are vulnerable to high temperatures.

The isolated bacterial strain was able to grow in the temperature range of 4–37°C, however, the optimal growth was observed at 15°C. The ribosomal RNA gene sequence of 1,447 bases obtained from IIIM-ST045 showed 99% homology with the corresponding gene sequences of *Stenotrophomonas* sp. The IIIM-ST045 was selected among 25 isolated bacterial strains for production of protease on the basis of clear zone formation on skimmed milk agar plate.

The effect of different carbon sources on alkaline protease production by *Stenotrophomonas* sp. was investigated. Among the various carbon sources used, lactose was found the best for production of protease enzyme with a specific activity of 157.50 U mg^{-1}. The effects of nitrogen source on alkaline protease production by *Stenotrophomonas* sp. including different inorganic, organic and cheap nitrogen sources were examined. The inorganic nitrogen sources like urea, ammonium chloride and ammonium sulphate showed the lowest alkaline protease productions of 38.9, 62.2 and 57.9 U mg^{-1} respectively. Interestingly, soya bean which is cheap nitrogen source showed the largest enzyme production of 398.65 U mg^{-1}.

In order to check the optimum alkaline protease production y *Stenotrophomonas* sp., five different media were tested at 15°C. The results indicate that Tryptic Soya broth (TSB) was the best medium for alkaline protease production with a specific activity of 87.2 U mg^{-1}. The time course of alkaline protease production showed that maximum enzyme production was observed at 32 h of incubation

Among different metals examined, Mg^{2+}, Mn^{2+} and Ca^{2+} showed the highest enzyme productions at

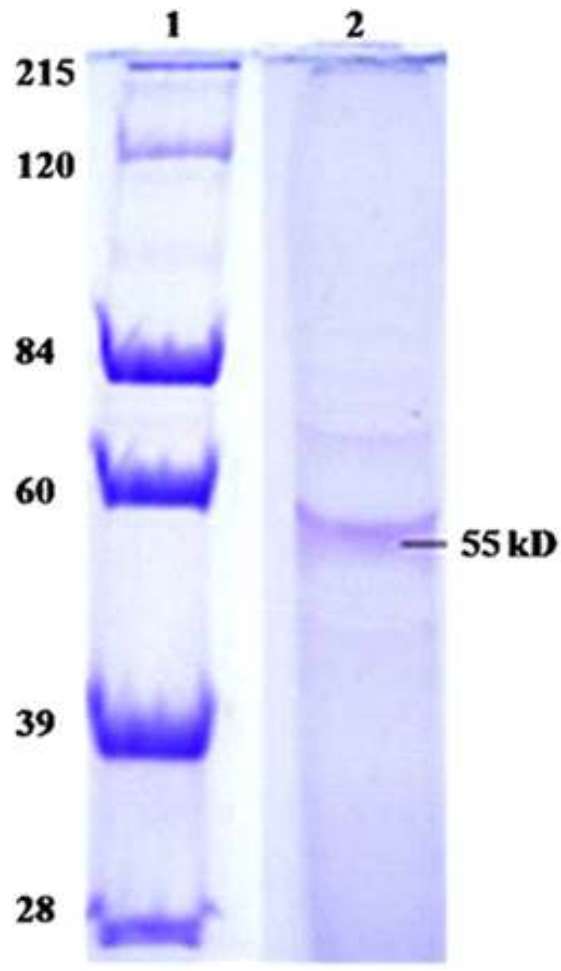


Fig. SDS-PAGE of purified protease: lane 1 molecular weight ladder; lane 2 partially purified protease

concentrations of 5 mM with relative activities of 110, 109 and 120% respectively). Incorporation of Mg^{+}, Mn^{+} and Ca^{+} together in growth medium showed higher enzyme production than using them individually. However, Zn^{2+}, Cu^{2+} and Co^{2+} strongly inhibited enzyme production.

Protease being extracellular was partially purified by ammonium sulphate precipitation followed by a series of chromatography steps in order to give an overall 18.45 folds purification with a specific activity of 41.2 U/mg of protein. The molecular weight of purified protease was calculated on the basis of semi logarithmic plots of the mobility of the band on SDS-PAGE using a standard curve established with proteins of known molecular weight. The molecular weight of the purified protease was determined to be ~55.0 kDa. The zymogram analysis after column purification showed a single band indicating that the protease is active as a monomer. The native molecular mass of the purified enzyme was in agreement with that obtained by zymogram gels. These results indicated that the protease is a monomeric enzyme.

The enzyme was active over a broad range of pH (6.8–12.0), showing maximum activity at pH 10.0. The effect of pH on stability of the enzyme was studied by using casein as a substrate under the standard assay condition. The pH-stability profile of the protease as determined by the residual activity measurement showed 90% of its original activity was retained between pH 6.8–12.0.

The enzyme displayed significant activity within a temperature range of 4–37°C with maximum activity at 15°C and at pH 10.0. In order to examine the temperature stability of the protease, the protease solution in 50 mM phosphate buffer (pH 7.0) was allowed to stand for 30 min at various temperatures. The enzyme retained more than 90% of its maximum activity after 1 h exposure to temperatures of 15–30°C and ~15–20% after 1 h exposure at a temperature of 40–50°C.

To check the enzyme and substrate concentration, the original stock enzyme preparation was diluted to eight different descending concentrations and was assayed for their activities. The results indicated that enzyme activity was directly proportional to the concentration of the enzyme used. Highest enzyme activity of 300.0 U/ml was observed at 0.4 U/mg of protein preparation. In case of the effect of the substrate on enzyme activity it is obvious from the results that the maximum protease activity (312.12 U/ml) was obtained when casein was used at a concentration of 1%. A higher concentration of casein (more than 1%) showed a dramatic decrease in protease activity with almost no activity at 10% casein as substrate.

Besides pH, a good detergent protease is expected to be stable in the presence of commercial detergents. The IIIM-ST045 protease showed excellent stability and compatibility in the presence of locally available detergents (Nirma, Wheel, Henko, Surf, Surf Excel, Ariel, and Rin). Protease from *Stenotrophomonas* sp. IIIM-ST045 showed stability and compatibility with a wide range of commercial detergents even at 15°C in the presence of $CaCl_2$ as stabilizer. Our protease showed good stability and compatibility in the presence of Ariel followed by other commercial detergents. The enzyme retained more than 50% activity with most of the detergents tested even after 3 h of incubation at 15°C. As the protease produced by our isolate was stable over a wide range of pH and temperature, which showed compatibility with various commercial detergents tested in the presence of $CaCl_2$. The supplementation of the enzyme preparation in detergent could significantly improve the cleansing of the stains.

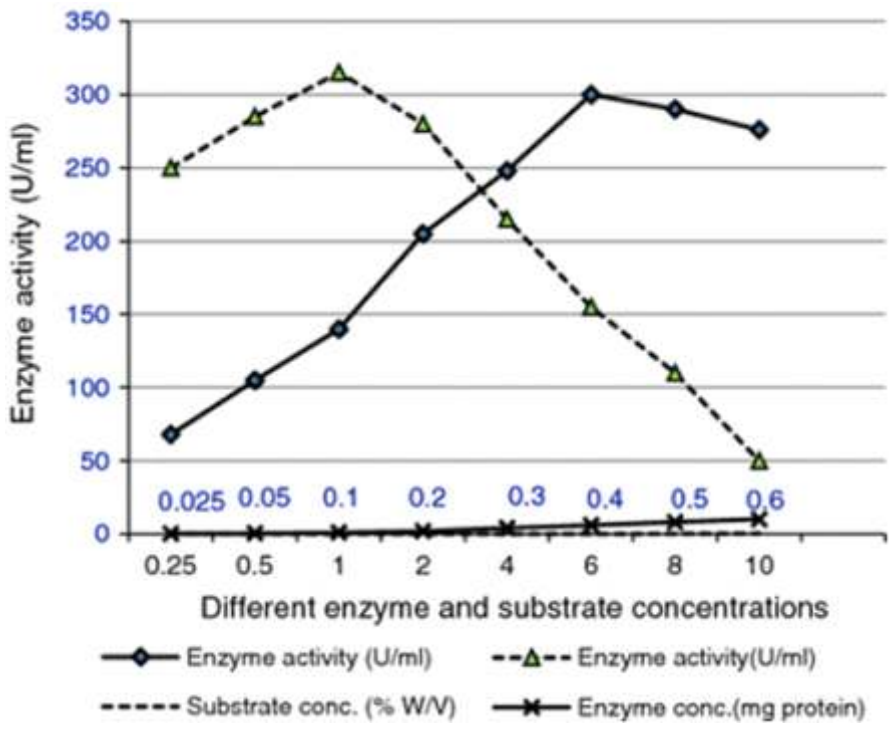


Fig. Effect of different enzyme and substrate concentrations on the enzyme activity of partially purified protease

10.2 Bacterial Diversity Of The Apharwat Glacier In North Western Himalayas Of Kashmir

Nasier Ahmad, Qazi Parvaiz Hassan and Sarojini Johri

The soil samples from the apharwat glacier in north western Himalayas of Kashmir, India were analyzed for the presence and diversity of bacteria by molecular phylogenetic analysis. The aim of this study was to describe the diversity of bacteria in apharwat glacier by 16S rDNA-dependant molecular phylogeny. Phylogenetic analysis suggested that the large amount of unclassified cloned sequences could imply that novel groups of bacteria were present in the glacier soil 16S rDNA library. The cloned DNA sequence similarity ranges of 82–99% were found in the 16S rDNA library. The results suggest that rDNA sequences linked to bacterial microflora probably represent many novel groups of bacteria. Total genomic DNA was isolated directly from soil using MoBio kit. Good quality, high molecular weight metagenomic DNA was isolated with the purity index of >1.8. Small subunit (SSU) rRNA gene sequences from bacteria were selectively amplified by PCR and cloned. Nucleotide sequence information of twenty eight clones obtained was used to determine the unique SSU rRNA gene sequences within the rDNA library.

All the one hundred ninety two (192) clones of bacterial rDNA were further analysed by restriction fragment length polymorphism (RFLP). Based on the RFLP pattern, redundancy of inserts was checked and clones with maximum variability in banding pattern were selected. A four-site cutter endonuclease *Taq* 1 was used for the digestion of the clones that resulted in 3–6 bands those were most suitable to score the genetic diversity. Twenty eight unique clones thus selected as a result of RFLP pattern were subsequently sequenced. Ten of the twenty eight cloned sequences when blasted showed significant homologies and subsequently under phylogenetic analysis with reference sequences obtained through BLAST analysis fell into five different phylogenetic groups representing Acidobacteria (14.3%), Nitrospira (10.7%), Chloroflexales (3.6%), Firmicutes (3.6%) and Proteobacteria (3.6%). While as eighteen of the cloned sequences did not prominently show significant homologies to any of the known bacterial phylas. All of these sequences reflected unique unknown phylotypes thereby showing the possible novelty of these domains. Similar results have been presented from bacterial diversity studies of different extreme locations all round the globe and novel bacterial strains reported.

Clone number	Closest match (Accession number)	Base pairs	%	Accession number	Phylogenetic group
AGK-004	Uncultured bacterium, EF018498.1	589	98	HQ834211	*Unknown*
AGK-009	Uncultured bacterium, EF516012.1	459	82	HQ834212	*Unknown*
AGK-014	Uncultured nitrospira, AJ519405.1	873	94	HQ834213	*Nitrospirae*
AGK-016	Uncultured acidobacteria, HM062461.1	1072	93	HQ834215	*Acidobacteria*
AGK-022	Uncultured bacterium, EF018065.1	1066	98	HQ834214	*Unknown*
AGK-028	Uncultured acidobacteria, EF019024.1	1066	99	HQ834216	*Acidobacteria*
AGK-033	Uncultured bacterium, JN024042.1	1070	98	HQ834217	*Unknown*

Clone number	Closest match (Accession number)	Base pairs	%	Accession number	Phylogenetic group
AGK-040	Uncultured chloroflexi, DQ450736.1	1075	92	HQ834218	*Chloroflexi*
AGK-048	Uncultured nitrospiraceae, EF018086.1	578	96	HQ834219	*Nitrospirae*
AGK-054	Uncultured nitrospira, AM167945	607	94	HQ834220	*Nitrospirae*
AGK-062	Uncultured bacterium, GQ339170	1123	92	HQ834221	*Unknown*
AGK-069	Uncultured acidobacteria, DQ450701.1	1083	94	HQ834222	*Acidobacteria*
AGK-074	Uncultured bacterium, FJ592799	1129	90	HQ834223	*Unknown*
AGK-082	Uncultured bacterium, GQ402748	1073	98	HQ834224	*Unknown*
AGK-091	Uncultured bacterium, EF492965	1135	95	HQ834225	*Unknown*
AGK-098	Uncultured bacterium, JF833538.1	1130	98	HQ834226	*Unknown*
AGK-106	Uncultured bacterium, FJ625318.1	1121	93	HQ834227	*Unknown*
AGK-125	Uncultured firmicutes, EF664979.1	620	95	HQ834228	*Fermicutes*
AGK-129	Uncultured bacterium, AY963478.1	615	94	HQ834229	*Unknown*
AGK-133	Uncultured bacterium, EF019080.1	700	99	HQ834230	*Unknown*
AGK-140	Uncultured bacterium, EU132364.1	1121	89	HQ834231	*Unknown*
AGK-144	Uncultured bacterium, GU598579	1072	99	HQ834232	*Unknown*
AGK-153	Uncultured bacterium, GQ339170.1	609	99	HQ834233	*Unknown*
AGK-160	Uncult. betaproteobacterium, FR749807	1107	98	HQ834234	*Proteobacteria*
AGK-168	Uncultured bacterium, DQ866136.1	594	91	HQ834235	*Unknown*
AGK-174	Uncultured bacterium, DQ866143.1	657	99	HQ834236	*Unknown*

Clone number	Closest match (Accession number)	Base pairs	%	Accession number	Phylogenetic group
AGK-186	Uncultured bacterium, EU470505.1	580	96	HQ834237	*Unknown*
AGK-192	Uncultured acidobacteria, DQ328617	690	97	HQ834238	*Acidobacteria*

Table. Similarity values of Bacterial 16S rDNA sequences retrieved from Apharwat glacier in north western Himalayas of Kashmir.

The snowpack is a diverse habitat that remains largely unexplored as research efforts have focused on glacier systems and ice. Many studies, including this one, report the occurrence of related phylotypes from geographically diverse, but predominantly cold environments, and the community structure has common populations in different cryohabitats. The bacterial diversity in this unique extreme habitat is being first time reported. The studies indicate the possible presence of novel groups of bacteria present in the glacier that may be further exploited for their better use during our further studies in drug discovery. Microbial ecosystems beneath glaciers and ice sheets have recently attracted significant attention due to their metabolic potential and possible role in regional and global carbon cycling and climate change. Although the molecular and Phylogenetic data collected in this study cannot help in inferring an ecological role for these microorganisms in the environment. The present study has made its contribution by providing data for further studies on the dynamics of bacterial populations in glacier regions of upper Himalayan range in north Kashmir.

List of Publications

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Patents filed

S.No.	Patent title	Inventors	NF Number
1.	Adaptogenic activity of extracts of *Eurycoma longifolia* (Tongkat Ali)	Bani Sarang , Pandey Anjali, Koul Surrinder, Sangwan Payarelal, M. Rajendran, Marnickavasagar V. (Malaysia Patent Dec. 2011).	US Patent 2012. (Filed by Dr. Rajen Holistica,Malaysia).
2.	Synthesis and biological evaluation of Isoform selective analogs of tetrazolyl-Quinazolinone scaffold as anticancer agents :P13K-alpha/beta inhibitors	Vishwakarma Ram Asrey, Sawant Sangapal, Singh Damodar, Pal Parvider, Dar Abid Hamid, Nargotra Amit, Sharma Parduman Raj, Saxena Ajit Kumar, Mahesuni Srinivas, Hussain, As hiq, Qazi Asif Khurshid, Mahajan Priya	150NF/2012
3.	Design, Synthesis and biological evaluation of isoform selective analogs of Liphagane scaffold as anticancer agents:P13K-alpha/beta inhibitors	Vishwakarma, R.A.; Sawant, S.D.; Singh, P.P.; Dar, A.H.; Sharma, P.R.; Saxena, A.K.; Nargotra, Amit; Kumar, K.A. Aravind; Mudududdla, Ramesh; Khursid, Asif; Hussain, Aashiq and Chanauria, Nayan	195NF/2011
4.	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	63NF/2012

5.	A novel formulation useful in Cancer chemotherapy.	Mondhe Dilip Manikrao, Taneja Subhash Chandera, Koul Surrinder, Dhar Jagdish Kumar, Saxena Ajit Kumar, Johri Rakesh Kamal, Wani Zahoor Ahmad, Andotra Samar Singh, Sharma Subash Chander, Singh Surjeet, Gupta Prem Narayan, Vishwakarma Ram Asrey	0088NF/2012

BOOKS

1. Natural Products: Research Reviews – Vol. 1,V.K.Gupta (Ed.). Daya Publishing House, New Delhi, 519p(2012)
2. Traditional and Folk Herbal Medicine: Recent Researches – Vol. 1 V.K.Gupta (Ed.). Daya Publishing House, New Delhi, 407p (2012)
3. Bioactive Phytochemicals: Perspectives for Modern Medicine – Vol. 1 V.K.Gupta (Ed.). Daya Publishing House, New Delhi, 516p(2012)
4. Medicinal Plants: Phytochemistry, Pharmacology and Therapeutics – Vol. 2 V.K.Gupta G.D.Singh, Surjeet Singh & A. Kaul (Ed.), Daya Publishing House, New Delhi. 433p (2012)
5. Animal Diversity, Natural History and Conservation – Vol. 1,V.K.Gupta, Anil K.Verma (Ed.). Daya Publishing House, New Delhi, 480p (2011)
6. Perspectives in Animal Ecology and Reproduction - Vol. 8 V.K.Gupta, Anil K.Verma, G.D.Singh, (Ed.). Daya Publishing House, New Delhi.494p (2011), Pages: 533

Book Chapter

1. Ajai Prakash Gupta and Suphla Gupta, "HPTLC–MS Coupling: New Dimension of HPTLC", High-Performance Thin-Layer Chromatography (HPTLC), Ed. M. M. Srivastava, Springer Heidelberg Dordrecht London New York, 331-353.
2. Ajai Prakash Gupta and Suphla Gupta (2012). Elemental Profiling: Its Role and Regulations, Atomic Absorption Spectroscopy, Dr. Muhammad Akhyar Farrukh (Ed.), ISBN: 978-953-307-817-5, InTech,

SEMINARS AND WORKSHOP

Poster presented:

1. I Khan, PL Sangwan, JK Dhar, S Koul (2011) Development of HPTLC method for simultaneous quantification of natural products from *Betula utilis*. Chemical Research Society of India North zone meeting, Sept. 22-24, 2011 Jammu University, Jammu PP-67.
2. P. Kumar, R. Reddy, S. Farooq, PL Sangwan, S Koul (2011) Anticancer activity of citral dimer derivatives. Chemical Research Society of India North zone meeting, Sept. 22-24, 2011 Jammu University, Jammu PP-66.
3. R Reddy, P. Kumar, S. Farooq, PL Sangwan, S Koul (2011) N-Iodosuccinimide: highly effective and regioselective reagent for iodoesterification of alkenes. Chemical Research Society of India North zone meeting, Sept. 22-24, 2011 Jammu University
4. Dr PL Sangwan attended 12th International Congress of Ethnopharmacology "Traditional Medicines and Globalization- The Future of Ancient System of Medicines" held at Jadavpur University, Kolkata, India during Feb. 17-19, 2012.

INVITED TALKS

Dr. S.Koul

1. In connection with refresher course for lecturers at GGM College, Jammu on Ist Oct'2011 on the topic ' SOME MEDICINAL ASPECTS OF NATURAL PRODUCTS
2. Exploring chemical reactions for biological processes in health and disease" at Govt. MAM College, University of Jammu (Sep 27th, 2011) during celebration of "2011 as International year of Chemistry"

Muzimil Ahmed

1 Role of Prostaglandin D2 in Cerebral Ischemia, National Seminar on Advances in Biosciences at Amar Singh College Srinagar on 12th June 2012.

2. Prostaglandin D2 in exacerbates Ischemic Injury, at XXXI Annual Conference of Society of Toxicology (STOX), December 22-24, 2011 at IIS University, Jaipur.

Abid Hamid Dar

1. Exploring chemical reactions for biological processes in health and disease" in context with "2011-The international Year of Chemistry". Organized by Govt. MAM College, University of Jammu and J & K Science & Technology on Sep 27th, 2011

RESEARCH COUNCIL

Prof. Goverdhan Mehta — Chairman
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Prof. Siddhartha Roy — Cluster Director
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Head or his Nominee — Permanent Invitee
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Dr. P.S. Ahuja — Member
Director, Institute of Bioresource Technology
Palampur

Dr. R.K. Raina — Member
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Technical Officer EI
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Sh. Amit Nargotra — Member
Scientist B
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Sh. Upendra Kumar — Member
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Sh. Om Prakash — Member
AO
Indian Institute of Integrative Medicine, Canal Road, Jammu

Externally Funded Projects

TITLE OF THE PROJECT	PROJECT LEADER	SPONSERED BY
Supply of Aromatic plants for R&D & extension	Dr.Suresh Chandra	Centre for Aromatic plants.Govt. of Uttarakhand
Engineering the apocartenoid biosynthetic pathway in saffron	Dr. Nasheeman Ashraf	Department of Science and Technology, (JK Govt.)
Identification & Molecular cloning of cytochrome P4 50 gene sequences from Colecus forskohlii	Dr. Sumit Gandhi	Department of Science and Technology, (JK Govt.)
Role of prostaglandin E2 receptor EP1 antagonist in stroke: mechanistic and pre-clinical approache	Dr. Muzamil Ahmad	Department of Biotechnology, New Delhi
Screening of some plant molicules Against Mycobacterium tuberculosis	Dr.Inshad Ali Khan	Indian Council of Medical Research, New Delhi

List of Fellowships Awarded

FELLOWSHIP	PROJECT LEADER	SPONSERED BY
INSPIRE Fellowship	Mr.Gurjinder Singh	Department of Science And Technology, New Delhi
ICMR Fellowship	Ms.Arpita Saxena	Indian Council of Medical Research, New Delhi
ICMR Fellowship	Mr.Mohd.Noor Alam	Indian Council of Medical Research, New Delhi
ICMR Fellowship	Harvinder Kour Khera	Indian Council of Medical Research, New Delhi
ICMR Fellowship	Arvind Raina	Indian Council of Medical Research, New Delhi

FELLOWSHIP	PROJECT LEADER	SPONSERED BY
DBT Fellowship(Ramalingaswami Fellowship)	Dr. Zahoor Ahmad Parry	Department of Biotechnology,New Delhi
INSPIRE Fellowship	Mytre Koul	Department of Science and Technology, New Delhi
ICMR Fellowship	Sakshi Malthotra	Indian Council of Medical Research, New Delhi
DBT Fellowship	Ankita Magotra	Department of Biotechnology, New Delhi
INSPIRE Fellowship	Vaibhav Khare	Department of Science and Technology, New Delhi
INSPIRE Fellowship	Ravinder Kumar Dhar Dubey	Department of Science and Technology, New Delhi
INSPIRE Fellowship	Ashok Kumar	Department of Science and Technology, New Delhi
INSPIRE Fellowship	Neha Sharma	Department of Science and Technology, New Delhi
ICMR Fellowship	Abid Manzoor Shah	Indian Council of Medical Research, New Delhi
INSPIRE Fellowship	Rohit Sharma	Department of Science and Technology, New Delhi
C.V. Raman International Fellowship For African Researchers	Dr. Anatole Constant Pieme	Department of Science and Technology, New Delhi
C.V. Raman International Fellowship For African Researchers	Dr. Pantaleon Ambassa	Department of Science and Technology, New Delhi
C.V. Raman International Fellowship For African Researchers	Dr. Kawthar Abdel Aziz EI-Sayed Mohd. Diab	Department of Science and Technology, New Delhi

Hkkjrh; leor vkÔ/k lLFkku] tEe e jkt HkkÔk dh ixfr e fgUnh dk ;Øe

llnh; jktHkkÔk lfefr dh vky[k ,o lk{; milfefr }kjk jktHkkÔk fujhÕ.k fnukd 23-04-2011 l 24-04-2011 dk uxj jktHkkÔk dk;kUo;u lfefr] tEe d v/;Õ rFkk dUnh; dk;ky;k@cdk@miØek d 15 lnL; dk;ky;k d lkFk jktHkkÔk uhfr dk;kUo;u lc/kh fopkj&foeÓ dk;ØeA

llnh; lfefr dh vkyk[k ,o lk{; mi lfefr }kjk jktHkkÔk fujhÕ.k dk;Øe

cBd e mifLFkr llnh; lfefr dh vky[k ,o lk{; milfefr }kjk jktHkkÔk fujhÕ.k fopkj&foeÓ dk;Øe fnukd 23-04-2012 l 24-04-2012 dk uxj jktHkkÔk dk;kUo;u lfefr dk;ky; ,o lfefr d vU; 15 dUnh; dk;ky;k@cdk@miØek d lkFk fopkj&foeÓ fd;k x;kA llnh; milfefr d ekuuh; mik/;Õ ,o ,o lnL;k] dk;ky; ie[kk dk Lokxr lLFkku d funÓd ,o v/;Õ] ujkdkl Mk- jke fo'odek u fd;kA bl volj ij Jh lR;or pronh] mik/;Õ] lkln jkT; lHkk] Jh j?koÓ ilkn flg] lnL;] ykd lHkk] Jh jktUn vxoky] lkln] ykd lHkk] Mk- ilUu dekj ikBlk.kh] lkln] ykd lHkk] Jherh iue tutk] lfpo] llnh; jktHkkÔk lfefr lfpoky;] Jh inhi dekj Óek] ofj- vul/kku vf/kdkjh] lfefr lfpoky;] Jh ohjUn flg] vul/kku lgk;d] lfefr lfpoky;] Jh xxk ilkn] fjikVj] lfefr lfpoky; rFkk dk;Øe e Ókfey ujkdkl lnL; dk;ky;k d dk;ky; ie[k mifLFkr FkA fopkj&foeÓ dk;Øe dh v/;Õrk llnh; lfefr d mik/;Õ Jh lR;or pronh u dhA fopkj&foeÓ d nkjku lHkk ifr egkn; u jktHkkÔk dk;kUo;u dk ln< cuku d fy, egRoi.k l>ko fn;A

Jh lR;or pronh u dgk fd ujkdkl dh cBdk e dk;ky; v/;Õ Lo; Hkkx ugh yr vkj mUgku vkifÙk 0;Dr djr g, fopkj&foeÓ dk;Øe e Ókfey lnL;k dk vkxkg fd;k fd ujkdkl dh cBdk e Hkkx u yu l cBd e fy, x; fu.k;k ij dkjokb lEHko ugh gk ikrhA xr rhu cBdk dh mifLFkfr d foÔ; ij xgjh fpUrk 0;Dr djr g, mifLFkr lnL;k l tkuuk pkgk fd vkidh jk"Vh; ,o iÓklfud ftEeokjh gA vkidk iÓklfud nkf;Rok dk fuokgu djr g, cBdk e vfuok; :i l mifLFkr jguk pkfg, vkj jktHkkÔk uhfr dk;kUo;u dk iHkkoh cuku dh fnÓk e vko';d dkjokb djuk pkfg,A

vUr e mifLFkr x.kekU; 0;fDr;k ,o lnL;k dk vkHkkj lfgr /kU;okn Mk- vkj-d-j.kk] ofj"B oKkfud u fd;kA

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Hkkjr ljdkj] xg e=ky;] jkt HkkÔk foHkkx d funÓkukj uxj jkt HkkÔk dk;kUo;u lfefr] tEe dh Nekgh cBd fnukd 29 tu] 2011 ¼c/kokj½ dk vijkgu 3-00 ct Hkkjrh; leor vkÔ/k lLFkku] tEe d dkUÝl gky e vk;kftr gbA cBd dh v/;Õrk lLFkku d funÓd ,o ujkdkl v/;Õ Mk- jke fo'odek u dhA bl volj ij e[; vfrfFk d :i e mifLFkr Jh foukn dekj Óek] mifunÓd ¼dk;k-½] Hkkjr ljdkj] xg e=ky;] jkt HkkÔk foHkkx] Õ=h; dk;kUo;u dk;ky; ¼fnYyh½] Jh ,l-lh-flg] dk;ikyd funÓd] ikoj fxM dkjikj'ku vkQ bfM;k fyfeVM ¼mRrjh Õ=&AA½] tEe] Jh jktho gLr] egkic/kd] ,u-,p-ih-lh- ifj;ktuk nygLrh fd'rokM] Jh lh-oh-flg] egkic/kd] ,u-,p-ih-lh- lyky ifj;ktuk] m/keij] Jh ijfoUnj flg] vk;Dr] lhek 'kYd ,o dUnh; mRikn ÓYd vk;Drky; ¼tEe ,o d'ehj½] Jh egkchj flg] l;Dr vk;dj vk;Dr] vk;dj dk;ky;] tEe] Jh vÓkd xlrk] mi egkic/kd] itkc uÓuy cd] Õ=h; dk;ky;] tEe] Jh vkeidkÓ] iÓklfud vf/kdkjh] Hkkjrh; leor vkÔ/k lLFkku] tEe] rFkk ujkdkl d dUnh; dk;ky;k@cdk@miØek d lHkh

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dk;ky;k/;Õ@fgUnh vf/kdkjh@fgUnh vuoknd@fiUV o byDVkfud ehfM;k d lHkh loknnkrk rFkk vU; x.kekU; 0;fDr mifLFkr FkA cBd dk lpkyu Mk- vej flg] ofj- fgUnh vf/kdkjh ,o lfpo] ujkdkl] tEe u fd;kAcBd dk lpkyu djr g, lLFkku d ofj-fgUnh vf/kdkjh ,o lfpo Mk- vej flg v/;Õ egkn; ,o cBd e mifLFkr lTtuk dk Lokxr Mk- vej flg] lnL;&lfpo u fd;kA mUgku viu Lokxr lck/ku e dgk] ^^fd ;g cBd iR;d Nekgh d nkjku Hkkjr ljdkj] xg e=ky;] jkt HkkÔk foHkkx d vknÓ ij cykb tkrh gA bl cBd e iFke vDVcj] 2010 l 31 ekp] 2011 d nkjku vkid dk;ky; e jkt HkkÔk fgUnh e fd; x; dk;k dh lehÕk rFkk jkt HkkÔk uhfr dk;kUo;u e mRiUu leL;kvk ij ppk dh tk,xhA mUgku dgk fd ujkdkl vki lcd fy, ,d lÓDr ep g ftld ek/;e l tEe d lHkh dUnh; dk;ky;k@cdk@miØek e jkt HkkÔk fgUnh d dk;kUo;u dh ixfr dh leh{kk vkj fnÓk ,o nÓk rFkk ljdkj d vknÓ@vunÓ lHkh dk;ky;k dk Hkt tkr g ftll vPNh idkj l dk;kUo;u ixfr dh vkj vxlj gk jgk gA mUgku vkÓk 0;Dr djr g, dgk fd lfefr oreku e vPN dk;kUo;u dh vkj mUe[k g vkj fudV Hkfo"; e ;fn vki lcdk lg;kx ikIr gvk rk ;g lfefr jkt HkkÔk d dk; fu"iknu e mRd"V Hkfedk vnk dju e lÕe gkxhA

cBd dk lpkyu djrs g, lLFkku dh ofj- fgUnh vf/kdkjh ,oa lfpo Mk- vej flg

lLFkku d funÓd ,o v/;Õ] ujkdkl] tEe Mk- fo'odek u mifLFkr lnL;k dk Lokxr djr g, viu v/;Õh; lck/ku e dgk] ^^fd 23 viy] 2011 d nkjku

llnh; jktHkkÔk lfefr dh milfefr }kjk jktHkkÔk fujhÕ.k fd;k x;k ftle Ókfey lnL; dk;ky;k dk foÓÔ lg;kx jgk ftld fy, lfefr dh vkj l mUgku lcdk ÓfØ;k vnk fd;kA fgUnh dk dEl;Vj d ek/;e l c<ku d fy, jktHkkÔk foHkkx d vknÓkulkj vkb-Vh- pfEi;u Vhe xfBr dju dk l>ko egRoi.k gA lHkh dk;ky;k dk dEl;Vj ij ;uhdkM d ek/;e l fgUnh dk; dk dju e mRiUu leL;kvk dk lek/kku lEHko gkxkA ftle mUgku nk&nk lnL; dUnh; dk;ky;k rFkk nk&nk cdk@miØek l puko dju dk iLrko fd;kA ;g Vhe lh-Md i.k d ek/;e l ifÓÕ.k ikIr djxh vkj ujkdkl lnL; dk;ky;k e dEl;Vj d ek/;e l ;fudkM ij ifÓÕ.k nxh lkFk gh lfpo] Hkkjr ljdkj] ub fnYyh d ek/;e l jk"V;kih Lrj ij ipkj&ilkj gr dk; ;ktuk d ek/;e l tk tykb&vxLr] 2011 d vUrxr ipkj&ilkj vfHk;ku ij nÓ e pyk;k tk;xk vkj v/;Õ] uxj lfefr }kjk Mh,ohih iuy e Ókfey dju d fy, v/;Õ egkn; u itkc uÓuy cd ¼Õ=h; dk;ky;½ tEe] Hkkjrh; LVV cd ¼iÓklfud dk;ky;½ tEe] dk;ky; egky[kkdkj] vk;dj dk;ky;] tEe] foekuiRru ikf/kdj.k] tEe o [kknh xkek|kx ¼dohvkblh½ tEe vkfn l Hkh nk&nk 0;fDr;k tk Mh,ohih iuy e Ókfey vkmV Mkj ipkj ,tfUl;k d ek/;e l lkotfud LFkyk ij ekxnÓh fl)kUr d vk/kkj ij jktHkkÔk foHkkx] ub fnYyh }kjk nh x;h ipkj&ilkj lkexh pfuUnk LFkku ij yxku dk dk; djx**A v/;Õ egkn; u lHkh lnL;k dk vkHkkj 0;Dr djr g, /kU;okn fd;kA

vUr e lLFkku d iÓklfud vf/kdkjh Jh vkeidkÓ u v/;Õ egkn; rFkk cBd e mifLFkr lHkh dUnh; dk;ky;k@cdk@miØek d dk;ky; ie[kk] ofj- fgUnh vf/kdkfj;k@fgUnh vuokndk ,o uxj d fiUV o byDVkfud ehfM;k d lHkh loknnkrkvk dk vkHkkj 0;Dr djr g, dgk fd ehfM;k dk lno bl cBd e fo'k"k lg;kx jgk gA e gn; l mudk vkHkkjh gA vki lHkh cBd e mifLFkr g, vkj mUgku lHkh lnL;k dk cgr&cgr vkHkkj lfgr /kU;okn fd;kA mUgku viu lLFkku d lHkh ldk; lnL;k dk cBd d vk;ktu e lfØ; lg;kx d fy, mudk Hkh /kU;okn lfgr vkHkkj 0;Dr fd;kA

vktknh dh 66oh oÔxkB

Lor=rk fnol dh 66oh oÔxkB dh volj ij jk"Vªiwt Qgjkrs g, lLFkku dh funÓd Mk- jke fo'odek A

vktknh dh 66oh oÔxkB d bl ikou volj ij lLFkku d funÓd Mk- jke fo'odek u lcdk gkfnd ÓHkdkeuk, nr g, dgk fd ;g ,frgkfld fnu ge Lor=rk vkUnkyu d gtkjk ijokuk dh egku dkfu;k dh ;kn fnyrk gA ge mu nÓ HkDrk] Óghnk rFkk nÓ dh jÕk e Óghn g, luk d tokuk o nÓ d ukxfjdk dk J)kleu vfir djr g ftUgku nÓ dh lhekvk ij jÕk djr g, viuh tku dcku dj nh g ejk mUg Ór&Ór i.kke !

15 vxLr] 1947 dk vkt l 65 oÔ igy ge vktkn g, FkA ;g gekjh vktknh dk foÓÔ egRoi.k ,o xkjoÓkyh fnu gA D;kfd 26 tuojh] 1930 dk jkoh unh d rV ij ug: th dh v/;Õrk e io Lor=rk ikflr dk iLrko dkxl d vf/koÓu e ikl gvk FkkA l?kÔ djr jg turk Lor=rk dh cfyonh ij p<rh jgh vkj Óghnk dk [ku jx yk;k vkj nÓ Lok/khu gvkA ge

Lok/khu g, 65 oÔ gk x; gA vc ;g le) Hkkjr g] geu bu 65 oÔk e D;k ik;k D;k [kk;k\ ge vkx vkj ixfr d fy, vkRe eUFku djuk gkxk] cgr lkjh miyfC/k;k geu gkfly dh gA foKku d Õ= e tk ixfr nÓ u dh g og dkfcy rkfjQ g] uohure vul/kku d lkFk&lkFk u;h&u;h nok, gekj nÓ e fodflr dh xb gA dfÔ d Õ= e vuitkÅ tehu dk dfÔ ;kX; cuku e dfÔ dh inkokj e of) gb gA bu 65 oÔk d nkjku vkfFkd fodkl nj] fonÓh enk HkMkj] [kk|kUu mRiknu] fo|r mRiknu] lkÕjrk nj vkfn e of) lHko g yfdu nÓ dh ljÕk d fy, Hkkjr etcrh d lkFk vkx c< jgk gA

funÓd egkn; u lLFkku dh Ók/k lc/kh oKkfud miyfC/k;k dk crkr g, ;ok oKkfudk dk bukofVo dh vkj c<u dk lnÓ fn;kA vk|kfxd Õ= e foKku d ek/;e l vk|kxhdj.k dh ifØ;k rt gb gA bldk vUnktk ge nÓ d lh-,l-vkb-vkj- lxBu ,o vU; oKkfud Ók/k lLFkku l egll dj ldr gA vkb-Vh- ,o i;koj.kh; leL;kvk d vfrfjDr vU; vkfFkd] mnkjhdj.k] lkekftd ,o jktufrd nf"Vdk.k dk 0;kid cukuk gA

vktknh gekj nÓ dh ,drk] v[k.Mrk dh irhd g gekj nÓ okfl;k dh ,d tVrk gh leUo;d lLdfr gA jk"Vfirk egkRek xk/kh] lHkkÔ pUn ckl] Hkxr flg] pUnÓ[kj vktkn] l[kno rFkk jktx: tl Lo=rk lukfu;k u ftu ckrk dk Qkyk dj dfBure y{; l Lor=rk ikIr dh mUgh d vknÓk ij pydj y{; ikIr dj vkx c<uk gkxkA e> viu u; fodflr lxfBr Hkkjr ij xo gA vkfFkd Lrj ij yxkrkj etcr gk jgk Hkkjr fodflr nÓ cuu dh drkj e vxlj gA yfdu bu lHkh miyfC/k;k dk J; mu egku Hkkjrh;k dk tkrk gA vr% bl ,frgkfld volj ij e tkfr] ox] HkkÔk] /ke vkj Õ= d rPN Hkn&Hkko l Åij mBdj ÓkfUr] lef) vkj ixfr d ekx ij pyr g, jk"Vh; ,drk o [k.Mrk dk etcr dju d fy, lHkh dk ,dtV gku dk ge ldYi yr gA

fgUnh fnol@llrkg] 2011 dk dk;Øe

fgUnh fnol@llrkg dh nkjku ifr;kfxrk e Hkkx yrs g, ifr;kxh o fu.kk;dx.k

vk;ktu dh x;h ftle fgUnh fucU/k] Jry[k] jktHkkÔk ,o foKku i'ukRrjh] vuokn@fVIi.k ,o ik:i.k] HkkÔ.k ifr;kxfrk] lkLdfrd dk;Øe vkfn ifr;kfxrk, vk;kftr dh xb rFkk fot;h ifr;kfx;k dk lLFkku d funÓd rFkk ujkdkl v/;Õ Mk- jke fo'odek u viu dj&deyk l udn jkfÓ rFkk iek.k&i= inku fd, x;A

l?k dh jktHkkÔk fgUnh e ljdkjh dkedkt rFkk fgUnh d ixfr :fp tkxfr dju d mnn'; l dUn ljdkj d dk;ky;k e fgUnh fnol@llrkg@ekl dk vk;ktu djuk lo/kkfud fu;ek d vulkj vfuok; gA pfd 14 fnlEcj] 1949 dk lfo/kku lHkk u fgUnh dk l?k dh jktHkkÔk d :i e vxhdkj fd;k FkkA rnulkj Hkkjrh; lfo/kku d vuPNn 343¼1½ e ;g mic/k fd;k x;k g fd fgUnh l?k dh jktHkkÔk gkxhA vr% jktHkkÔk foHkkx }kjk okfÔd dk;Øe d vulkj gh ;g vk;ktu djuk okfNr gA Hkkjrh; leor vkÔ/k lLFkku e fgUnh fnol@llrkg] 2011 dk vk;ktu fnukd 07 flrEcj] 2011 l 14 flrEcj] 2011 rd rrlc/kh ifr;kfxrk, fuEu dk;Øekulkj

ifr;kfxrk e Hkkx yrs g, ifr;kxh ,oa fu.kk;d eMy dh lnL;

fgUnh dk;Ókyk

dk;Ókyk dk lapkfyr djrs gq, lLFkku dh funÓd Mk- jke fo'odsd ,oa lHkh LVkQ lnL;

l?k dh jktHkkÔk fgUnh e ljdkjh dkedkt rFkk fgUnh d ixfr :fp tkxfr dju d mnn'; l dUn ljdkj d dk;ky;k e fgUnh fnol@ llrkg@ekl d volj ij fgUnh dk;Ókyk dk vk;ktu fd;k x;kA ftle lLFkku d funÓd Mk- jke fo'odsd u viu fopkj 0;Dr djr g, dgk fd iÓklu d lHkh LVkQ lnL;k ,o rduhdh vf/kdkfj;k dk Hkh tgk rd lHko gk fu"Bkiod viu oKkfud dk;k e fgUnh dk i;kx djuk pkfg,A rkfd oKkfud ,o iÓklfud Õ= e fgUnh dh ixfr lfuf'pr gk ldA

Mk- vkj-d-j.kk] v/;Õ] ih-,e-b- u viu fopkj 0;Dr djr g, dgk fd oKkfud cU/k ,o iÓklu d lHkh LVkQ lnL; ;fn i;kl dj rk fgUnh dk i;kx fu%lng c<k;k tk ldrk gA

Jh miUn dekj] foRr ,o y[kk vf/kdkjh u viu fopkj 0;Dr djr g, dgk] ^^fd iÓklu d lHkh Õ=k e fgUnh dk;kUo;u dk c<k;k tk ldrkA lLFkku d iÓklfud vuHkkxk e gh fgUnh dh dk; idfr dk c<ku dk ek/;e gA tcfd Hkkjr ljdkj] xg e=ky;] jktHkkÔk foHkkx d vknÓ g fd iÓklu] foRr ,o y[kk rFkk Hk.Mkj ,o Ø; vuHkkxk e leLr dk; fgUnh e lEiUu dju d fy, ikf/kdr fd;k gA

vUr e Mk- vej flg] ofj"B fgUnh vf/kdkjh u dk;Ókyk e lHkh lg;kxh LVkQ lnL;k dk vkHkkj lfgr /kU;okn fd;kA

uxj jktHkkÔk dk;kUo;u lefr] tEe dh Nekgh cBd fnukd 25 tuojh] 2012 dk Hkkjrh; leor vkÔ/k lLFkku] tEe d dkUÝl gky e lEiUuA

Hkkjr ljdkj] xg e=ky;] jktHkkÔk foHkkx d funÓkulkj uxj jktHkkÔk dk;kUo;u lefr] tEe dh Nekgh cBd fnukd 25 tuojh] 2012 ¼c/kokj½ dk vijkgu 2-30 ct Hkkjrh; leor vkÔ/k lLFkku] tEe d dkUÝl gky e vk;kftr gbA cBd dh v/;Õrk lLFkku d funÓd ,o ujkdkl v/;Õ Mk- jke fo'odsd u dhAbl volj ij Jh ijfeUnj flg] vk;Dr] lhek ÓYd ,o dUnh; mRikn ÓYd] tEe] Jh ,l-lh-flg] dk;ikyd funÓd] ikoj fxM dkjikjÓu] tEe] Jh vÓkd xlrk] miegkic/kd] itkc uÓuy cd] eMy dk;ky;] tEe] Jh vkj-,u-eh.kk] eMy ;krk;kr ic/kd] mRrj jyo] tEe] Mk- vkj-d-j.kk] ofj"B oKkfud] Hkkjrh; leor vkÔ/k lLFkku] tEe d

v/;Õ] ujkdkl Mk- jke fo'odeh cBd dh lkekf/kr djrs gq,

ujkdkl] tEe d dUnh; dk;ky;k@cdk@miØek d lHkh dk;ky;k/;Õ@fgUnh vf/kdkjh@fgUnh vuoknd] fiUV o byDVkfud ehfM;k d lHkh loknnkrk rFkk vU; x.keU; O;fDr mifLFkr FkA

loiFke cBd e mifLFkr lTtuk dk Lokxr Mk- vej flg] ofj- fgUnh vf/kdkjh ,o lfpo] ujkdkl] tEe u fd;kA mUgku viu Lokxr lck/ku e dgk] ^^fd bl cBd e iFke viy] 2011 l 30 flrEcj] 2011 d nkjku lHkh dk;ky;k l ikSr ixfr fjikV dh leh Õk dh tk;xhA fgUnh dk;kUo;u l lcf/kr leL;kvk ij fopkj&foeÓ fd;k tk;xkA cBd dk e[; mnn'; g fd dUnh; dk;ky;k e fgUnh dh ixfr dl lEHko gk l?k d fofHkUu jktdh; i;ktuk e bld ixkeh i;kx dk c<kok nu d fy, jktHkkÔk foHkkx ifr oÔ ,d okfÔd dk;Øe tkjh djrk g] ftld vulkj ge dk;ky;k e jktHkkÔk d dk; lEiUu djr gA pfd ljdkjh dkedkt e ey fVli.k vkj ik:i.k d fy, fgUnh dk gh i;kx fd;k tk,A ftld vUrxr /kkjk 3½3½ dk ge lcdk vuikyu lfuf'pr djuk pkfg,A Hkkjrh; lfo/kku e tk iko/kku fd; x; g blh d vulkj ge vknÓk@vunÓk dk ikyu djr g, egkefge jk"Vifr d ldYik dk lEeku djuk pkfg,**A rRi'pkr cBd e mifLFkr lnL;k d ifjp; d lkFk gh cBd dh dkjokb vkjEHk gbA lnL;&lfpo u xr cBd d dk;oRr ij ppk d nkjku dgk fd lEekuuh; lnL;k dh dkb ifrfØ;k] l>ko vFkok vkifÙk gk rk di;k viu fopkj j[k yfdu ekuuh; mifLFkr lnL;k dh vkj l dkb vkifRr ,o ifrfØ;k u feyu ij lfpo u v/;Õ egkn; d vueknu ij xr cBd d dk;oRr dh if"V dhA

ujkdkl] tEe dh xg if=dk Kkuokrk vad 2 dk foekpu djrs gq, lLFkku funÓd ,oa v/;Õ] ujkdkl Mk- jke fo'odeh ,oa vU; lnL; dk;ky;k dh dk;ky; ie[k

ujkdkl] tEe dh cBd e ^Kkuokrk* d vd f}rh; dk foekpu fd;k Xk;k lkFk gh v/;Õ egkn; u vU; lnL; dk;ky;k dh jktHkk"kk xg if=dkvk dk Hkh inÓu fd;k vkj lnL;k e forfjr dh x;hA

lnL;&lfpo] Mk- vej flg u lfefr dk crk;k fd ekp] 2012 d vfUre llrkg e v/;Õ] ujkdkl] tEe dk;ky; ,o dk;ikyd funÓd] ikoj fxM dkjikjÓu] Õ=&AA] tEe] dk;ikyd funÓd] ,u-,p-ih-lh-Õ=&A] tEe dk;ky;k d ek/;e l vf[ky Hkkjrh; dfo lEeyu] ;fudkV ij ifÓÕ.k dk;Øe vk;kftr fd;k tk;xkA bl ckj e tYn gh lcf/kr dk;ky;k l fopkj&foeÓ fd;k tk;xkA jktHkkÔk foHkkx] Õ=h; dk;kUo;u dk;ky; fnYyh d ek/;e l njHkkÔ ij gb okrk d vulkj mRrj Õ= d ujkdkl dk;ky;k d v/;Õ@lfpok dk Hkh jktHkkÔk lEeyu e vkef=r dju dk l>ko gA ;fn mudk fyf[kr :i l iLrko v/;Õ egkn; dk vk;k rk v/;Õ ujkdkl dk;ky; d vueknu l ;g dk;Øe Hkh vk;kftr fd;k tk;xkA

oÔ 2010&2011 d nkjku dUnh;

oÔ 2010&2011 dh nkjkujktHkkÔk uhfr dh J"B fu"iknu dh fy, ÓhYM ikIr djrh gq, lLFkku dh funÓd Mk- jke fo'odeky

dk;ky;k@cdk@miØek dk jktHkkÔk uhfr d J"B fu"iknu d fy, jktHkkÔk ijLdkj ftle ¼1½ Hkkjrh; leor vkÔ/k lLFkku] tEe ¼2½ vk;Dr] lhek ÓYd ,o dUnh; mRikn ÓYd vk;Drky;] tEe ¼3½ vk;on Õ=h; vul/kku lLFkku] tEe ¼4½ itkc uÓuy cd] eMy dk;ky;] tEe o d'ehj ¼5½ Hkkjrh; LVV cd] iÓklfud dk;ky;] tEe ¼6½ bMfLV;y MoyieV cd vkQ bfM;k ¼vkb-Mh-ch-vkb-½] Ók[kk] tEe ¼7½ ikoj fxM dkjikjÓu vkQ bfM;k fyfeVM] tEe ¼8½ ,u-,p-ih-lh-fyfeVM] Õ=h; dk;ky; ¼Õ=&A½] tEe ¼9½ Hkkjrh; foekuiÙku ikf/kdj.k] tEe vkfn dk;ky;k dk ÓhYM ,o iek.k&i= v/;Õ] ujkdkl] tEe Mk- jke fo'odek u inku fd,A blh ifji{; e fgUnh vf/kdkfj;k@fgUnh vuokndk ,o fgUnh loh dfe;k dk v/;Õ egkn; u efjV iek.k&i= inku fd;A

lLFkku d funÓd ,o v/;Õ] ujkdkl tEe Mk- jke fo'odek u lHkkxkj e mifLFkr uxj d dk;ky; ie[kk ,o vU; x.kekU; 0;fDr;k dk uooÔ dh ÓHkdkeuk, nr g, viu v/;Õh; lck/ku e dgk] ^^fd uxj jktHkkÔk dk;kUo;u lfefr ^x* Õ= e gkr g, lHkh lnL; dk;ky;k e jktHkkÔk d dk;k e ixfr lfuf'pr gb g vkj geu viu lLFkku e fgUnh dh egRoi.k iLrd viu iLrdky; e vyx l 0;ofLFkr <x l l/kh ikBdk d v/;;u gr ic/k fd;k gA vki Hkh bll ykHkfUor gk ldr g ejk lHkh lnL; dk;ky;k l vujk/k g fd vki Hkh viu&viu dk;ky;k e KkuihB l ijLdr]

fgUnh d vuokn@vPN lkfgR; dh iLrd [kjhnu dh 0;oLFkk dj rkfd fgUnh d i;kx dk c<kok feyA mUgku viu egRoi.k l>ko e dgk fd ,d xi vkb-Mh-b-ey cukb tk;xhA ftle lHkh lnL; dk;ky; ie[kk d vkb-Mh-l lEid fd;k tk ldrk gA cBd ,o vU; jktHkkÔk lc/kh xfrfof/k;k cM&cM dk;ky;k e vk;ktu dju dk l>ko fn;kA flrEcj ekl d nkjku vkid dk;ky;k e cgr vPN&vPN dk;Øe fgUnh d i;kx dk c<ku dh fnÓk e fd, x; g] bll lkfcr gkrk g fd gekj nÓ e fgUnh d i;kx ,o vU; Hkkjrh; HkkÔkvk d ÓCnk dk vkRelkr dju rFkk tEe&d'ehj jkT; dh lkLdfrd fofo/krk dk HkkÔkvk d ek/;e l infÓr dju dk ,d ekx iÓLr gvkA ;g ge tEe&d'ehj e n[ku dk feyk g gekj ujkdkl dh ^Kkuokrk* xg if=dkvd% 2 vkid leÕ iLrr gA ble igy dh viÕk vf/kd btkQk gvk g ble y[k ,o jpuk, lUnj Óyh e fyfic) dh x;h gA vkx Hkh vkid lg;kx l Kkuokrk d vd vkj lUnj cuku d i;kl jgxA

vUr e lLFkku d ofj"B oKkfud Mk-vkj-d-j.kk u lLFkku d funÓd ,o ujkdkl] v/;Õ Mk- jke fo'odek th dk foÓÔ vkHkkj 0;Dr djr g, dgk fd mUgku oKkfud dk;k d vfrfjDr cBd d vk;ktu gr viuk egRoi.k le; fn;k bld fy, ge mudk /kU;okn djr g lkFk gh ØeÓ% Jh ijfeUnj flg] vk;Dr lhek ÓYd ,o dUnh; mRikn ÓYd] Jh ,l-lh-flg] dk;lkyd funÓd] ikoj fxM dkjikjÓu fyfeVM] tEe] Jh vÓkd xlrk] miegkic/kd] itkc uÓuy cd½eMy dk;ky;½ tEe] Jh vkj-,u-eh.kk] eMy ic/kd ;krk;kr] mRrj jyo] tEe u viuk egRoi.k le; fudkydj cBd dk ,d ub fnÓk nh ge mud vR;f/kd vkHkkjh g rFkk ujkdkl tEe d lHkh dUnh; dk;ky;k@cdk@miØek d dk;ky; ie[kk] fgUnh vf/kdkfj;k@fgUnh vuokndk ,o uxj d fiUV o byDVkfud ehfM;k d lHkh loknnkrkvk jfM;k d'ehj] njnÓu dUn] tEe u cBd d dcjt dju e viuk egRoi.k lg;kx inku fd;kA ge mudk foÓÔ vkHkkj 0;Dr djr gA lLFkku d lHkh ldk; lnL;k dk ftUgku cBd d vk;ktu gr fofHkUu idkj l viuk i.k lg;kx inku fd;k g rFkk lLFkku d ofj- fgUnh vf/kdkjh ,o lnL; lfpo] Mk- vej flg rFkk leLr LVkQ lnL;k dk vkHkkj lfgr /kU;okn fd;k A

x.kr= fnol lekjkg

x.kr= fnol dh 62oh oÔxkB dh ikou volj ij lLFkku d funÓd Mk- jke fo'odek u mifLFkr lHkh x.kekU; 0;fDr;k ,o lHkh LVkQ lnL;k dk gkfnd ÓHkdkeuk, nr g, dgk fd vkt d fnu gekj nÓ dk lfo/kku ykx gvk FkkA 26 tuojh] 1950 dk geu ,d vUrfojk/kk d thou e ioÓ fd;k FkkA ge x.kr= fnol ij dN u; ldYi yu gkx ftu ij vey dj vki ,d vPN ukxfjd cu jg rkfd vki jk"V fuek.k e vkidh Hkfedk jgA fdlh ykdrkf=d] itkrkf=d nÓ e fof/k ekuo thou dk vk/kkj gkrk g tcfd fdlh nÓ d dkuu dk Kku lHkh ukxfjdk dk gk rkfd o vud idkj d vijk/kk l cp ldA Hkkjr e ub fof/k 0;oLFkk ,o ekfyd vf/kdkjk dh i.k LFkkiuk lfo/kku e 26 tuojh] 1950 dk ykx gku d lkFk gk pdh Fkh] gkykfd mldk vkfÓd :i e ikjEHk 26 uoEcj] 1949 dk gh gk x;k Fkk tcfd lkekftd] vkfFkd] U;k;kf;d rFkk jktufrd nf"V l Hkkjrh; ijEijk e ;g ,d u; ;x dk l= ikr gvk] D;kfd gekjh fof/k 0;oLFkk e o dky dkuu vkj vekufÔd 0;ogkj fufÔ) gk x; Fk]

x.kr= fnol dh 62oh oÔxkB dk volj ij lcksf/kr djrs g, lLFkku dk funÓd Mk- jke fo'odek

ftUgku vkneh dh xfjek ifr"Bk rFkk eku&lEeku dk u"V&Hk"V dj fn;k FkkA

lfo/kku fof/k dk lc/k ik;% fdlh nÓ d Óklu ,o dkuu 0;oLFkk l gkrk gA lfo/kku fyf[kr ik:i] iy[kk ;k fu;ek] ykdkpkjk] ijEijkvk vkj 0;ogkjk ij vk/kkfjr gk ldrk gA ble mu fofo/k fu;ek dk lxg gkrk g ftud vulkj ml nÓ dh Óklu 0;oLFkk lpkfyr dh tkrh gA og Óklu d ljpukRed ,o dk;kRed iÕk dk foLrr Lo:i rFkk lxBu fu/kkfjr djrk gA

lfo/kku dk fuek.k lfo/kku lHkk dh iFke cBd 9 fnlEcj 1946 dk vkjEHk gb rc ;g mEehn ugh Fkh fd Mk- vEcMdj dh Hkfedk bruh lkFkd ,o fu.kk;d jgxh ftruh dh dkykrj e fl) gbA bUg lfo/kku ik#i ¼Mk¶V½ lfefr dk v/;Õ cuk;k x;k ftUgku u; lfo/kku dh leLr fuek.k ifØ;k e mud fopkj&foeÓ rFkk ekfyd lÓk/ku e egRoi.k fu.k; Lo; fy, vkj lfo/kku lHkk l ikfjr djok, lfo/kku 25 uoEcj 1949 dk cudj r;kj gvk rc mud io vud lnL;k u Mk- vEcMdj dh ckf)d Õerk] yxu] Kku] lgHkkfxrk rFkk jpukRed Hkfedk dh foÓÔ ljkguk dh vkj mUg lfo/kku dk e[; fÓYidkj rFkk vk/kfud eu dh lKk nhA fu%lUng Mk- vEcMdj lfo/kku d e[; fuekrk funÓd dg x;A

vkj-vkj-,y-gkbZ Ldwy dh cPpk }kjk lkLdfrd dk;Øe dh ,d >yd

oreku lfo/kku vkj x.kr= dh me vkt jkr 12 ct 61 oÔ i.k gkxA fdlh x.kr= dh lQyrk@vlQyrk d fy, 61 oÔ dh vof/k de ugh gkrhA vktkn d igy 562 fj;klr] jkT; jtokM Fk] ljnkj iVy u viu i;klk l mUg Hkkjrh; jk"V e feyk;kA vkt gekjh lhek, fQj Hkh vÓkr g iokÙkj jkT;k e ck:nh xn g tgk vkUrfjd ljÕk [krj

e g rFkk fonÓh Hkh gekj ixfr ,o fodkl d fy, xHkhj ugh gA jktufrd] lkekftd] ckf)d rFkk lkLdfrd Õ=k e bu vfHkykÔkvk dk i.k dj ld tgk vÓkd pØ dkuu 0;oLFkk d /kepØ dk ifrfuf/kRo djrk g ogh Hkxok jx R;kx dk irhd g rFkk gjk jx feVVh iM&ik/kk l gekj tMko dk irhd g ftl ij lEi.k thou fuHkj g ge vkRekuÓklu vkj lR; d fy, lQn jx l funfÓr gkuk pkfg, blh idkj ge lcdk lxfBr ,o Hkkod gkdj jk"V&fuek.k e ;kxnku nuk gkxkA

vkt gekjk Hkkjr c<r fonÓh enk Hk.Mkj] lpuk ik|kfxdh d Õ= e foKku ixfr d fy, fodflr nÓk dh Hkkfr mUufr dh nkM e vkx vk;k g ogh 0;kikj vk|kfxdhdj.k vkj fuoÓ e c<kRrjh gb g yfdu vkx Hkh ixfr dh nkM e vkx c<uk gA fu%lUng Hkkjr vkfFkd nf"V l lEiUu gkrk g rk oKkfud Õ= e vul/kku d fy, vkfFkd <kp dk vkj vf/kd etcr dj ldxkA ge iÓu dju dk dke dju dk tTck ftld cy ij ;ok Økfrdkfj;k u vktknh fnyku d fy, lg;kx fn;k FkkA vkt ifrLink dk ;x g ge ifr;kxh cudj lQyrk ikIr dj ldr g ge fujkÓ ugh gkuk pkfg, ;g eoeUV ge fl[kkrk g fd ge fujUrj i;kl dju gkx lpuk vkj ik|kfxdh] dfÔ] LokLFk] fÓÕk foKku d Õ= e Hkkjr vkx c<k gA

vr% bl ,frgkfld volj ij tkfr] ox] HkkÔk] /ke vkj Õ=h;rk d rPN Hkn&Hkko l Åij mBdj ÓkfUr] lef) vkj ixfr d ekx ij pyr g, jk"Vh; ,drk o v[k.Mrk dk etcr dju d fy, Hkkjrh; lfo/kku e inRr ykd dY;k.kdkjh ÓfDr;k dk etcr cukuk gA

uxj jktHkkÔk dk;kUo;u lfefr] Hkkjrh; leor vkÔ/k lLFkku] tEe d rRoko/kku e vk;dj dk;ky;] tEe ,o lhek ÓYd ,o dUnh; mRikn ÓYd] tEe d lktU; l jktHkkÔk lEeyu@;fudkM ifÓÕ.k dk;Øe

ifÓÕ.k dk;Øe dk mn?kkVu djrs g, lLFkku d funÓd ,oa v/;Õ] ujkdkl Mk- jke fo'odek A

lLFkku d funÓd ,o v/;Õ] ujkdkl Mk- jke fo'odek AifÓÕ.k dk;Øe dk mn?kkVu 29 ekp] 2012 dk ikr% 9-30 ct vkb-vkb-vkb-,e-] tEe d dkUÝl gky e dk;Øe dk ÓHkkjEHk Mk- Jhd".k fuey] mifÓÕk vf/kdkjh] ub fnYyh }kjk ljLorh onuk xk;u l lLFkku d funÓd Mk- jke fo'odek u nhi&iTToyu dj dk;Øe dk mn?kkVu fd;kA rRi'pkr i"i&xPN l tEe d vk;dj vk;Dr Jh jfo lkjxy u ujkdkl v/;Õ Mk- jke fo'odek dk Lokxr fd;kA Mk- vej flg] ofj- fgUnh vf/kdkjh ,o lnL;&lfpo] ujkdkl] tEe u lHkkxkj e mifLFkr fofÓ"V vfrfFk;k ,o ifrHkkfx;k dk Lokxr fd;kA

uxj jktHkkÔk dk;kUo;u lfefr Hkkjrh; leor vkÔ/k lLFkku tEe d rRoko/kku e vk;dj dk;ky;] tEe ,o lhek ÓYd

;fudkM ifÓÕ.k dk;Øe dk lckf/kr djrs g, lLFkku dh funÓd ,oa v/;Õ] ujkdkl Mk- jke fo'odek A

,o dUnh; mRikn ÓYd] tEe lktU; l fnukd 29&30 ekp] 2012 dk jktHkkÔk lEeyu@;fudkM ifÓÕ.k dk;Øe dk vk;ktu fd;k x;kA bl ifÓÕ.k dk;Øe e ujkdkl tEe d dUnh; dk;ky;k@cdk@miØek rFkk lLFkku d vf/kdkfj;k@depkfj;k ,o jktHkkÔk l lc) inkf/kdkfj;k u Hkkx fy;k ftle oDrkvk d foÔ; fuEu idkj g%& jktHkkÔk d dk;kUo;u e ;fudkM dh mi;kfxrk] fgUnh d dk;kUo;u e ;fudkM dh leL;k ,o lek/kku] jktHkkÔk dk;kUo;u e HkkfÔd lEiÔ.k ,o 0;kogkfjd Lo:i] jktHkkÔk uhfr d ifji{; e ;fudkM

lhek ÓYd ,oa dUnh; mRikn ÓYd] tEe@Jhuxj dh vk;Dr Jh ijfeUnj flg lkMh viu fopkj 0;Dr g,

dh lkFkdrk] l?k dh jktHkkÔk vkj gekjk nkf;Ro] oKkfud ,o rduhdh lLFkkuk e fgUnh y[ku dh Hkfedk vkfn foÔ;k ij vkef=r 0;k[;krkvk u viu 0;k[;ku iLrr fd,A

dk;Øe dh v/;Õrk lLFkku d funÓd ,o v/;Õ] ujkdkl tEe Mk-

jke fo'odek u dhA bl volj ij vk;dj dk;ky;] tEe@Jhuxj d vk;Dr Jh jfo lkjxy] lhek ÓYd ,o dUnh; mRikn ÓYd] tEe@Jhuxj d vk;Dr Jh ijfeUnj flg lkMh rFkk lLFkku d ofj- oKkfud Mk- vkj-d-j.kk Hkh mifLFkr FkA

tEe d vk;dj vk;Dr] Jh jfo lkjxy u lHkkxkj e mifLFkr lTtuk ,o ifrHkkfx;k dk Lokxr djr g, viu lck/ku e dgk fd ;g dk;Øe uxj jktHkkÔk dk;kUo;u lfefr Hkkjrh; leor vkÔ/k lLFkku] tEe ep d ek/;e l vk;kftr fd;k tk jgk g bld ij.kkikr lLFkku d funÓd vkj ujkdkl d v/;Õ Mk- jke fo'odek th dk gkfnd c/kkb nr g, mUgku dgk fd mud lg;kx l uxj tEe d lHkh dUnh; dk;ky;k e jktHkkÔk d dk;kUo;u e egRoi.k btkQk gvk gA mud fnÓk&funÓ egRoi.k g] pfd o ,d oKkfud gkr g, Hkh jktHkkÔk dh ixfr e mud i;kl mRd"V gA mUgku lHkh ifr;kfx;k dk ifÓÕ.k yu vkj jktHkkÔk d dk;k e ;kxnku dju d fy, vkàku fd;kA

Jh ijfeUnj flg lkMh]

vk;Dr lhek ÓYd ,o dUnh; mRikn ÓYd] tEe@Jhuxj u viu lck/ku e v/;Õ egkn; ,o mifLFkr lTtuk dk Lokxr djr g, dgk fd ;g ifÓÕ.k dEl;Vj l lcf/kr g D;kfd iR;d dUnh; dk;ky;k e dEl;Vj d ek/;e l dk; dju dk okrkoj.k cuk g vkj bl ifÓÕ.k d ek/;e l ;fudkM dh leL;k vkj mld lek/kku d fy, ;g ifÓÕ.k mi;kxh gA eu Lo; vuHko fd;k g fd tEe ujkdkl ep d ek/;e l jktHkkÔk dh mUufr d fy, vPN dk;Øe vk;kftr fd; tk jg gA mUgku v/;Õ

tEe ,oa d'eh dh vk;dj vk;Dr Jh jfo lkjxy viu fopkj 0;Dr g, A

egkn; dk vkHkkj 0;Dr djr g, lHkh ifr;kfx;k dk ,l ifÓÕ.k dk;Øek e Hkkx yu l Lo; mudh dk; dju dh Õerk e of) gkrh gA

;fudkM ifÓÕ.k dk;Øe d lekiu volj ij v/;Õ] ujkdkl Mk- jke fo'odek u lHkh ifÓÕ.kkfFk;k dk iek.k i= inku fd;A

/kU;okn iLrko lLFkku d ofj"B oKkfud Mk- vkj-d-j.kk u lLFkku d funÓd ,o v/;Õ ujkdkl tEe Mk- jke fo'odek th u bl ifÓÕ.k dk;Øe e viuk veY; le; fn;k vkj ;g dk;Øe mUgh d ekxnÓu l lEiUu gvkA e mud ifr vkHkkj 0;Dr djrk gA Jh jfo lkjxy vk;dj vk;Dr] tEe u bl dk;Øe e i/kkj dj rFkk vkfFkd :i l dk;Øe d vk;ktu e viuk ;kxnku fn;k ge mud cgr gh vkHkkjh g Jh ijfeUnj flg lkMh] vk;DrA bl ifÓÕ.k e l;Dr :i l vkfFkd lg;kx inku fd;k

tEe ,oa d'ehj dk vk;dj vk;Dr Jh jfo lkjxy viu fopkj 0;Dr g, A

lLFkku dk funÓd ,oa v/;Õ ujkdkl Mk- jke fo'odek u ;fudkM ifÓÕ.k dk;Øe e ifrHkkfx;k dk iek.k i= inku djrs g,A

vkj viuk mi;kxh le; fn;k ge mud foÓÔ vkHkkjh gA mUgku u ifÓÕ.k e mifLFkr okg; ldk; d :i e vkef=r 0;k[;kudrkvk@ifrHkkfx;k rFkk byDVkfud ,o fiUV ehfM;k d lHkh loknnkrkvk dk /kU;okn fd;kA dk;Øe d l;ktd Mk- vej flg] lnL;&lfpo] ujkdkl] tEe ftUgku bl ifÓÕ.k dk;Øe d vk;ktu e foÓÔ ifjJe dj dk;Øe lQy cuku e viu nkf;Rok dk fuokgu fd;k rFkk ge viu lLFkku d lHkh lg;kxh c/kvk dk vkHkkj 0;Dr djr g] ftud ekxnÓu ,o lg;kx l ;g dk;Øe cgrj <x l lEiUu gvkA

Hkkjrh; leor vkÔ/k lLFkku] tEe d rRoko/kku e ikoj fxM dkjikjÓu] Õ=&AA] tEe ,o ,u,pihlh] Õ=&A] tEe d lktU; l vf[ky Hkkjrh; dfo lEeyu

vf[ky Hkkjrh; dfo lEeyu dh v/;Õrk djrs g, lLFkku dk funÓd ,oa ujkdkl v/;Õ Mk- jke fo'odek A

uxj jktHkkÔk dk;kUo;u lfefr] Hkkjrh; leor vkÔ/k lLFkku] tEe d vkfMVkfj;e gky Ek 30 ekp] 2012 dk lk; 5-30 ct e vf[ky Hkkjrh; dfo lEeyu dk vk;ktu fd;k x;kA dfo lEeyu dh v/;Õrk lLFkku d funÓd ,o ujkdkl] v/;Õ Mk- jke fo'odek u dhA mifLFkfr Jkrkvk ,o jk"Vh; dfo;k dk Lokxr Mk- vej flg] ofj-fgUnh vf/kdkjh ,o lnL;&lfpo o l;ktd u fd;kA ftle jk"Vh; Lrj d uk dfo;k dk vkef=r fd;k x;kA loiFke Mk- jke fo'dek u ek ljLorh d Lo:i ij ekY;ki.k dj ijEijkxr nf"V l nhi&iTToyu dj dfo lEeyu dk mn?kkVu fd;kA rRi'pkr jk"Vh; lr dfo Mk- cgknj flg funkÔh u i"i&xPN l dfo lEeyu d v/;Õ Mk- fo'odek th dk Lokxr fd;k ,o Jh ,l-d- cly] e[; ic/kd] ikoj fxM dkjikjÓu] tEe }kjk v/;Õ egkn; dk Óky HkV dj lEekfur fd;kA rRi'pkr Mk- jke u dfo lEeyu d lpkyu dk dk;Hkkj jk"Vh; lr dfo Mk- cgknj flg funkÔh dk lkikA Mk- funkÔh th u mifLFkr dfo;k dk i"iekykvk l Lokxr fd;kA vUr e mifLFkr dfo;k dk lLFkku d funÓd ,o lEeyu d v/;Õ Mk- jke fo'odek u lHkh dk Óky ,o Lefr&fpUg HkV fd;A jk"Vh; lr dfo Mk- funkÔh u Mk- fo'odek th dk Lefr&fpUg HkV dj lEekfur fd;kA

lLFkku d funÓd ,o dfo lEeyu d v/;Õ Mk- jke fo'odek u viu v/;Õh; lck/ku e mifLFkr jk"Vh; dfo;k ,o Jkrkvk dk Lokxr djr g, viu lck/ku e dgk fd ,l jk"Vh; Lrj

d dfo lEeyu vki lcd fy, vkj gekj fy, ,d ;knxkj cudj jgxA mifLFkr dfo;k dh ok.kh mudh iLrfr gekj eu dk rk iHkkfor fd;k gh g mudh jpukvk u ge ,d fnÓk nhA ge /;kueXu gkdj dfo;k dh jpukvk dk lur g vkj euu djr g] fopkjr g fd gekj fy, dju ;kX; minÓkRed lnÓ D;k g\

D;kfd ge oKkfud g vkj geu dfo;k d }kjk iLrr mudh lHkh jlk l ;Dr dk0; mfDr;k dk jlkLoknu fd;kA mUgku vkàku djr g, ep l ?kkÔ.kk dh fd ;g dk;Øe volj vku ij iu% vk;kftr fd;k tk;xkA

vUr e /kU;okn iLrko lnL;&lfpo Mk- vej flg] l;ktd u v/;Õ

egkn; dk vkHkkj 0;Dr djr g, dgk fd dfo lEeyu d ekxnÓu d fy, cgeY; le; fn;k lkFk gh dfo;k ,o Jkrkx.kk dk vkHkkj lfgr /kU;okn fd;kA

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Mr Bhushan Lal
Mr. Nirmal Singh
Mr. OM Singh
Mr. Parshotam Kumar
Mr. T.S. Salathia
Mr. Jasbir Singh
Mr. Ravinder Wali
Mrs .Kamlesh Sharma
Mrs. Manju Sambyal
Mrs. Neelam Sharma
Mr. A. K. Sharma
Mr. Parshotam Kumar
Mr. Madan Lal
Mr. Kuldeep Singh
Mr. Rajinder Kumar Gupta
Mrs . Sunita Devi
Mr . R. L. Jolly
Mr. A.K. Mehra
Mr. Vikram Bhardwaj
Mrs. Parveen Sharma
Dr. Ravinder Kour
Mrs. Shabnam Khan

Technician
Mr. Pushap Rattan
Mr. Girdhari Lal
Dr. Anil Prabhakar
Mr. Ashwani Sharma
Mr. Partap Chand
Mr. S.k. Ganjoo
Mr. Samar Singh
Mrs. Kiran Koul
Mr. Satya Bhushan
Mrs. Sarla Bhat
Mr. Rajinder Kumar
Mr. Naresh Pal
Ms. Anjum Vashist
Mr. Rajesh Kumar Sahdev
Mr. Yasir Arafat Chowdhary
Mr. Surinder Kumar

Lab Assist.
Mrs. Santosh Baigra
Mr. Dilbag Rai
Mr. Jita Ram
Mr. Shimlu Ram
Mr. Girdhari Lal
Mr. Gulam Quadir Sheikh
Mr. Chaman Lal
Mr. Bishan Kumar
Mr. Jasbir Singh
Mr. Kuldeep Kumar
Mr. Madan Lal
Mr. Moti Ram
Mr. Nasibu Ram
Mr. Sham Lal Bhagat
Mr. Sham Lal
Mr. V.P. Kohli
Mr. Balwant Raj
Mr. Hens Raj
Mr. Kartar Chand
Mr. Sham Lal
Mr. Ram Pal
Mr. Balwant Raj
Mr. Babu Ram
Mr. Dila Ram
Mr. Gudu Ram
Mr. Abid Hamid Dar
Mr. Karam Chand
Mr. Wali Wani
Mr. Mohd. Wani.
Mr. Rasool Mir
Mr. Neel Kamal
Mr. Rishi Kumar
Mr. Balwinder Singh
Mr. Manoj Kumar
Mr. Ajit Ram
Mr. Lal Chand
Mr. Om Parkash
Mr. Girdhari Lal
Mr. Abdul Ahad Sheikh
Mr. Fayaz Ahmad Dhar
Mr. Bushan Lal
Mr. Naranjan Singh
Mrs. Darshana
Mr. Nagar Lal
Mr.Kuldeep kumar

Admn. OfficerGr(1)
Mr. Om Parkash

Finance & Accounts Officer
Mr. Upendra Kumar

Store & Purchase Officer
Mr. Ashok Kumar

Sr. Hindi Officer
Dr. Amar Singh

Hindi Officer
Dr. Rama Sharma

Section Officer
Mr. S.R. Alam
Mr. Rajesh Kumar Gupta

Section Officer(Store & purchase)
Mr. B.B. Gupta

Private Secretary
Mr. Ramesh Kumar

Section Officer(F & A)
Mr. Anil Gupta
Mr. Darshan Singh

Security Officer
Mr. Yashpal Singh

Assistant General Gr(1)
Mrs. Vijay Bazaz
Mr. Major Singh
Mr. Anil Kumar Gupta
Mr. Romesh Kumar Mottan
Mr. U.S. Thappa

Mrs. Kusum Bali
Mrs. Neelam Razdan
Mr. Ranjeet Kr. Gupta
Mr. Manoj Kumar
Ms. Nisha Vij
Mr. Rajinder Singh
Mrs. Kiran Dutta

Asst.(F&A) Gr(1)
Mr. Tarsem Lal
Mr. Umesh Malhotra
Mr. H.K Gupta

Asst.(S&P) Gr(1)
Mr. Ram Singh
Mr. Satish Sambyal
Mr. Y.K. Mishra
Mrs. Rajni Kumari
Mr. Ashok Kumar

Senior Stenographer
Mr. V.K. Sharma
Mrs. Phoola Kumari

Security.Asstt.
Mr. Mohan Lal
Mr Krishan Lal

Receptionist
Ms. Jyoti Prabha

Asstt. (G)Gr(1)
Mrs. Rekha Gupta

Asstt (F&A) Gr(II)
Mr. Vinod Kumar Meena
Mrs. Lovely Ganjoo.
Mrs. Saroj Mehta
Mr. Sanchit Kumar Sharma

Asstt (S&P) Gr(II)
Mr. Bua Ditta

Asstt (G) Gr(III)
Mr. Benjamin
Mr Mohd Ayub Bhat

Asstt (F&A) Gr(III)
Mr. Roshan Lal

Asstt (S&P) Gr(III)
Mr. Angraz Chand
Mrs. Sunita Kumari

Staff Car driver
Mr. Bachitar Singh
Mr. Ashok Kumar
Mr. Kewal Singh
Mr. Kasturi Lal
Mr. Girdhari.

Record Keeper
Mr. Tilak Raj
Mr. Amar Nath

Halwai
Mr. Janak Raj

Work Assist.
Mr. G. M. Mir
Mr. Milkhi Ram
Mr. Paras Ram
Mr Sham Lal
Mr. Panna Lal
Mr Rahim Mir
Mr. Abdul Rahim Dar.(Mistry)
Mr. Jagdish Singh
Mr. Romesh Kumar
Mr. Chaman Lal
Mr. Parshotam Lal
Mr. Mohd. Farooq Bhat
Mr. Banadic Hans
Mr. Ram Lal
Mr. Ashok Kumar
Mr. Tarseem Kumar
Mr. Pawan Kumar
Mr. Rajesh k. Tandon
Mr. Moses Tegi
Mr.Girdhari Lal.
Mr Sodhagar Mal
Mr. Rashpal
Mr.Prithvi Raj
Mr. Mangal Dass
Mr. Sham Lal
Mr. Subash Chander
Mrs. Ratna
Mr. Girdhari lal
Mr. Suram Chand
Mr. Bala Ram
Mr. Tara Chand
Mr. Rattan Lal
Mr. Sham Lal
Mr. Kala Ram
Mr. Ashok Kumar
Mrs. Satya Sharma
Mr. Bua Ditta
Mr. Kehar Singh
Mr. Sat Pal
Mrs. Ram Pyari
Mr. Seva Ram
Mr. Sodagar Lal
Mr. Madan Lal
Mr. Ram Ditta
Mr. Sodagar Mal
Mr. Krishan Chand
Mr. Noor Mohd. Dar
Mr. Balwant Raj
Mr. Ashok Kumar
Mr. Munna
Mr. Dev Raj
Mr. Surinder Kumar
Mr. Ashok Kumar
Mr. Karnail Chand
Mr. Bachan Lal
Mr. Kali Das

Blood donation camp organized at IIIM, Jammu

Director inaugurating the IIIM Foundation Day celebration weak at the Badminto Court

Director shaking hands with the players of IIIM cricket team playing a friendly match with the J&K Armed Police

The chief guest Dr C. M. Gupta Distinguished Scientist being welcomed by Dr Rm Vishwakarma on CSIR-IIIM Foundation Day

Dr. S. K. Brahmachari, DG CSIR meeting the representatives of the Pharma Industry at Jammu

Hon'rable CM of J&K state Mr Omar Obdullah on his visit to IIIM, Jammu and interacting with the Scientists andStudents of the institute

Director Dr Ram Vishwakarma explaining the activities of the institute

Chief Minister Laying the Foundation Stone of the modern Animal House at IIIM

First Meeting of Scientific Advisoy Committee to Chief Minister being held at IIIM

Chief Minister releasing the Vision Document on 'Harnessing Biodiversity and Biotechnology for Progress of Jammu and Kashmir State'

CSIR Programme on Youth for Leadership in Science (CPYLS)