Appendices

Appendix A: Chemicals and equipment

Table 1 Chemicals and reagents used in the current work and their manufacturers

Item	Manufacturer/Supplier
General chemicals	Sigma – Aldrich (USA), Chromadex (USA), Merck Ltd (India), Himedia (India), Bangalore Genei (India), S D Fine Chemical Limited (India)
Molecular biology kits	
GenElute™ Gel Extraction Kit	Sigma-Aldrich Inc., USA
GenElute™ Plasmid Miniprep Kit	Sigma-Aldrich Inc., USA
GenElute PCR cleanup kit	Sigma-Aldrich Inc., USA
PCR-select™ cDNA SSH kit	Clontech, USA
SMARTer™ RACE cDNA Amplification Kit	Clontech, USA
Superscript® III first-strand synthesis system for RT – PCR	Invitrogen, USA
TA cloning kit	Invitrogen, USA
The QIAexpressionist™ System	Qiagen, Germany
Enzymes	
Advantage® 2 Polymerase mix	Clontech, USA
Restriction enzymes	Thermo Fisher Scientific Inc., USA
Reverse transcriptase	Invitrogen, USA
Taq polymerase	Qiagen, Germany
SYBR green master mix	Applied Biosystems, USA

Vectors	
pCR@2.1 (TA cloning kit)	Invitrogen, USA
pQE30 (The QIAexpressionist™ System)	Qiagen, Germany
DNA/RNA/Protein markers	
DNA markers	Thermo Fisher Scientific Inc., USA
RNA markers	Thermo Fisher Scientific Inc., USA
Protein markers	Sigma-Aldrich Inc., USA
Bacterial strains/competent cells	
One Shot® TOP 10 competent cells	Invitrogen, USA
M15 (pREP4) cells (The QIAexpressionist™ System)	Qiagen, Germany
Techware	
Microtips and micro-centrifuge tubes	Tarsons Products Pvt. Ltd. (India)
PCR tubes	Axygen, USA
MicroAmp fast optical 96 – well reaction plate (for RT – PCR)	Applied Biosystems, USA
Petriplates, Oak ridge tubes	Tarsons Products Pvt. Ltd. (India)
Glassware	Borosil Glass Works Ltd. (India)
Membrane filters	Himedia, India

Table 2 List of apparatus/euipments used in the current work

Apparatus/equipment	Model	Brand
Autoclave	VX – 75	Systec (Germany)
Centrifuge (s)	Eppendorf Mastercycler pro S, Thermo Scientific Sorvall Biofuge Primo R	Eppendorf (Germany), Thermo Fisher Scientific (USA)
Column (for HPLC)	X-bridge™ amide column (4.6 mm x 150 mm, i.d. 3.5 µm)	Waters Technologies (Ireland Limited, Ireland)
Cryocan (s)	BA-3, BA-20	Indian Oil (India)
DNA/RNA electrophoresis Unit (s)	Sub-cell®GT, Mini sub® cell GT	Bio – Rad Laboratories (USA)
Gel documentation system	ChemiDoc™ XRS⁺ Imaging system	Bio – Rad Laboratories (USA)
Hot air oven	Lab companion OF – 22G	Jeio Tech (South Korea)
High performance liquid chromatography (HPLC)	Dionex UltiMate 3000 BioRS HPLC System	(Thermo Scientific, USA)
Ice making machine	SPR – 80	Simag (Italy)
Incubator shaker	Innova 42R incubator shaker	New Brunswick Scientific Co., Inc. (USA)
Laminar airflow	Purifier Vertical Clean Benches	Labconco (USA)
Low temperature freezer (-20 °C)	Sanyo Biomedical freezer, MDF – U537D	Sanyo Electric Biomedical Co Ltd (Japan)
Milli Q water purifier	Milli-Q® Academic	Merck Millipore (Germany)
Nanodrop spectrophotometer	Nanodrop 2000c	Thermo Scientific (USA)
pH meter	PB-11	Sartorius (Germany)
Pipette (s)		Eppendorf (Germany), Tarsons Products Pvt. Ltd. (India), CAPP
Power packs for	PowerPac™ Basic,	Bio – Rad Laboratories

DNA/RNA electrophoresis unit Mini sub® cell GT		(USA)
Power packs for DNA/RNA electrophoresis unit Sub-cell®GT	PowerPac™ HC	Bio – Rad Laboratories (USA)
Power packs for protein electrophoresis unit PROTEAN®II xi cell	PowerPac™ Universal Power Supply	Bio – Rad Laboratories (USA)
Protein electrophoresis unit	PROTEAN®II xi cell	Bio – Rad Laboratories (USA)
Realtime PCR	StepOne Plus	Applied Biosystems
Refrigerator	-	Godrej
Spectrophotometer	UV – 1800	Shimadzu (Japan)
Thermal cycler	Eppendorf Mastercycler pro S	Eppendorf, Germany
Ultra-low temperature freezer (-80 °C)	5812	Thermo Fisher Scientific (USA)
UV transilluminator	Bentop 3UV™ transilluminator	UVP (USA)
Vacuum pump	Rockyvac 300	Tarsons (India)
Vortex	Spinix-vortex shaker	Tarsons (India)
Water bath	Lab companion RW – 1025G	Jeio Tech (South Korea)
Water purification system	Surepro prefiltration system/Elix water purification system	Merck Millipore (Germany)
Weighing balance	BSA224S-CW	Sartorius (Germany)

Standard experimental procedures

I. DNA extraction from gel

DNA extraction using GenElute™ Gel Extraction Kit (Sigma-Aldrich Inc., USA): An empty 2 ml centrifuge tube was weighed and its weight was recorded as W1. The tube with gel containing the amplicon was recorded as W2. The gel weight, W was calculated as:

W = W2 – W1. Three volumes of gel weight of gel solubilization solution was added to the tube and heated at 60 °C in a water bath for 10 min with intermittent tapping every 2 – 3 min. While the gel wasbeing solubilized, a column preparation was done by placing a column into 2 ml collection tube and adding 500 µl of column preparation solution to it. The column was then centrifuged at 16,000 x g for 1 min and the flow through was discarded. One gel volume of 100 % isopropanol was added to the solubilized solution of gel and mixed. It was then loaded onto column, 700 µl at a time and centrifuged for 1 min. The column was washed with 700 µl of wash solution and centrifuged at 16,000 x g for 1 min. The column was centrifuged again at 16,000 x g for 1 min without adding anything to remove excess ethanol. The column was then transferred to a fresh collection tube and 10 – 20 µl (depending on the intensity of the amplicon band) of elution solution pre – heated to 65 °C was added to the center of the column. The column was centrifuged at 16,000 x g for 1 minto get the eluate. DNA concentration in the eluate was quantified using NanoDrop™ 2000c spectrophotometer (Thermo Fisher Scientific Inc., USA). The DNA was stored at – 20 °C until required.

II. Plasmid isolation

A GenElute™ Plasmid Miniprep Kit (Sigma-Aldrich Inc., USA) was used to isolate the plasmids from *E. coli* transformants according to the manufacturer's protocol as follows: a single colony of transformants was grown in LB broth containing 0.1 µg/µl of ampicillin at 37 °C at 200 RPM shaking overnight. After using 750 µl of this overnight culture to make 50 % glycerol stock, the remaining culture was transferred to microcentrifuge tubes and pelleted by centrifugation at 12000 x g for 01 min. The pellet was resuspended using 200 µl of resuspension solution by pipetting up and down to make a homogeneous mixture. The cell lysis was performed using 200 µl of lysis solution and mixing the contents by gentle inversion 6 – 8 times and then keeping the lysis reaction at room temperature 5 min. 350 µl of neutralization solution was added and the contents were mixed by gentle inversion 6 – 8 times. The cell debris was then pelleted by centrifugation at 12000 x g for 10 min. 500 µl of column preparation solution was added to the GenElute Miniprep Binding Column and centrifuged at 12000 x g for 01 min. The cleared lysate was added to the column and centrifuged at 12000 x g for 1 min. The flow – through was discarded. Then 750 µl of diluted (ethanol added) wash solution was added to the column and centrifuged at 12000 x g for 01 min. The column was centrifuged again at 12000 x g for 02 min without adding any additional wash solution. The column was transferred to a fresh

collection tube and 20 – 50 µl of elution solution warmed at 50 – 60 °C was added to the column. The column was then centrifuged at 12000 x g for 1 min to elute the plasmid. The concentration and purity of plasmid was checked using NanoDrop™ 2000c spectrophotometer (Thermo Fisher Scientific Inc., USA) as mentioned in section 3.4 and then stored at – 20 °C until required.

III. Quantitation and electrophoresis of RNA

The quantitation and purity of RNA was checked using NanoDrop™ 2000c spectrophotometer (Thermo Fisher Scientific, USA). The ratio of absorbance at 260 and 280 nm was used to determine the purity of RNA. A value in the range of 1.8 – 2.0 was considered ideal for the use of RNA for further works. The integrity of RNA was checked through 1% denaturing agarose gel electrophoresis (Appendix B). 1 µg of RNA was mixed with RNA loading dye (Appendix B) and heated at 65 °C for 10 minutes, kept on ice for 2 minutes and then electrophoresed at 72 volts in 1X MOPS buffer (Appendix B) (Sambrook and Russell 2001) in a mini – sub cell GT electrophoresis unit (Bio-Rad, USA).

IV. Quantitation of DNA and plasmids

The quantitation and purity of DNA and plasmids were checked using NanoDrop™ 2000c spectrophotometer (Thermo Fisher Scientific, USA). The ratio of absorbance at 260 and 280 nm was used to determine their purity. A value in the range of 1.8 – 2.0 was considered ideal.

V. PCR clean up

PCR products were purified using GenElute PCR cleanup kit (Sigma-Aldrich Inc., USA) as follows: GenElute mini spin column was placed in a collection tube and 500 µl of column preparation solution was added. The column was centrifuged at 12000 x g for 30 s and the flow through was discarded. Five volumes of binding solution was added to the PCR product and mixed. The mixture was then loaded to the binding column and centrifuged at 12000 x g for 1 min and the flow through was discarded. 500 µl of wash solution was added and centrifuged at 12000 x g for 1 min. The flow through was discarded. The column was centrifuged again at 12000 x g for 2 min without adding additional wash solution. The flow through was discarded. The column was transferred to a fresh tube and 50 µl of elution solution was added. The column was centrifuged at 12000 x g for 1 min. The eluate was quantified using nanodrop spectrophotometer and was stored at – 20 °C until

further use.

VI. Preparation of chemically competent cells

Competent DH5α cells and M15 (pREP4) cells were prepared using the protocol described in The QIAexpressionist^{TM}, 5th edition (Qiagen, Germany) as follows: DH5α/M15 cells were streaked on a Luria Bertani (LB) medium and incubated at 37 °C overnight. A single colony from this plate was inoculated in 10 ml LB broth in a 100 ml conical flask and grown at 37 °C overnight with 200 RPM shaking in an innova 42R incubator shaker (New Brunswick Scientific Company, USA). 1 ml of this overnight grown culture was added to 100 ml prewarmed LB broth in a 250 ml conical flask and grown at 37 °C with 200 RPM shaking in an incubator shaker until an A_{600} of 0.5 was reached. The culture was cooled on ice for 5 min and transferred to two precooled 50 ml Oak Ridge tubes. The tubes were then centrifuged at 4000 x g for 5 min at 4 °C in Sorvall Biofuge Primo R (Thermo Scientific, USA). The supernatant was discarded and the cells were kept on ice. The cells were resuspended in 30 ml of ice cold transformation buffer 1 (TFB1) and kept on ice for 90 min. The cells were then centrifuged at 4000 x g for 5 min at 4 °C. The pellet was resuspended in 4 ml ice cold transformation buffer 2 (TFB2). The competent cells were then snap frozen in liquid nitrogen in aliquots of 100 and 200 μl and then stored in – 80 °C until required.

VII. Preparation of 12 % SDS – PAGE gel

SDS – PAGE was conducted using 12 % separating gel and 4 % stacking gel. The separating gel was prepared first by adding the reagents as shown below. The solution was degassed using a vacuum pump for 10 – 15 minutes and then poured in the gel cast. The gel was allowed to solidify for 30 – 40 minutes and then stacking gel was poured on top of the separating gel and allowed to solidify for 30 – 40 minutes. The stacking gel was prepared by adding the reagents as shown in the table below. It was also degassed using a vacuum pump for 10 – 15 minutes.

Reagents	12 % Separating gel (0.375 M Tris, pH 8.8)	4 % Stacking gel (0.125 M Tris, pH 6.8)
Acrylamide/bis-acrylamide (30 %)	16.00 ml	1.3 ml
ADW	13.40 ml	6.1 ml
1.5 M Tris.Cl, pH 8.8	10.00 ml	NA
1.5 M Tris.Cl, pH 6.8	NA	2.5 ml
10 % (w/v) SDS	0.40 ml	100 µl
10 % (w/v) APS (freshly prepared)	200 µl	50 µl
TEMED	20 µl	10 µl
Total	40 ml	10 ml

Appendix B: Preparation of reagents

Ampicillin (100 mg/ml): Dissolve 100 mg of ampicillin in 1 ml of ADW and filter sterilize through 0.22 µm filter. Store in aliquots at -20 °C.

Colony lysis buffer: 10 mM TE (pH 8.0), 0.1 % tween 20

Competent cell preparation reagents:

- **Transformation buffer 1 (TFB1):** 100 mM RbCl, 50 mM manganese chloride $MnCL_2$, 30mM CH_3CO_2K, 10 mM $CaCl_2$, 15 % glycerol. Adjust the pH to 5.8, filter sterilize using 0.45 µm filter and store at 4 °C.
- **Transformation buffer 2 (TFB2):** 10 mM MOPS, 10 mM RbCl, 75 mM CaCl2, 15 % glycerol. Adjust pH to 6.8 with 5 M KOH and filter sterilize

Denaturing formaldehyde agarose gel (1 %, 100 ml): Dissolve 1 g of agarose in 20 ml of 5X MOPS buffer and 65 ml of DEPC treated water by heating. Cool the flask to about 50 °C and then add 15 ml of formaldehyde solution. Add 5 µl of ethidium bromide (10 mg per ml), mix and pour into gel tray. Allow the gel to solidify for 30 min.

DNA loading dye (6X; 0.25 % bromophenol blue, 0.25 % xylene cyanol FF, 30 % glycerol): To make 10 ml of 6X DNA loading dye, mix 0.025 g each of bromophenol blue and xylene cyanol FF in little amount of water, add 3 ml of glycerol and make up the volume to 10 ml with ADW. Store the dye at – 20 °C.

EDTA, 0.5 M, pH 8: Add 93.06 g of EDTA in 400 ml of ADW. Adjust the pH to 8 using 10 N NaOH. Make up the volume to 500 ml and autoclave.

Gel running buffer for RNA (5X): Dissolve 20.9 g of MOPS in 800 ml DEPC treated water and add 6.66 ml of 3 M sodium acetate (pH 5.2). Adjust pH to 7.0 with 2 N NaOH and then add 20 ml 0.5 M EDTA (pH 8.0). Make up the volume to 1.0 liter with DEPC treated water.

Note: 1X MOPS – 20 mM MOPS, 4 mM sodium acetate, 2 mM EDTA.

IPTG (100 mM): Dissolve 238.31 mg of IPTG in 10 ml ADW and filter sterilize using 0.22 µm filter. Store at -20 °C in aliquots.

Kanamycin (25 mg/ml): Dissolve 25 mg of kanamycin in 1 ml of ADW and filter sterilize through 0.22 µm filter. Store in aliquots at -20 °C.

Luria broth: The composition of Luria broth – Casein enzymic hydrolysate (10 g/l), yeast extract (5 g/l), sodium chloride (10 g/l).

Luria Bertani (LB) medium plates: Use Luria Bertani agar, Miller with composition – Casein enzymic hydrolysate (10 g/l), yeast extract (5 g/l), sodium chloride (10 g/l), agar (15

g/l) to prepare LB plates. Autoclave the required volume of LB medium with the above composition and allow to cool to approximately 50 °C. Dispense the medium into sterile petri plates in laminar air flow cabinet and allow it to solidify. After covering with the lid, keep the petri plates in inverted position overnight at 37 °C in an incubator. Then store the plates at 4 °C after wrapping with aluminum foil.

Psi broth: Luria Bertani (LB) medium, 4 mM magnesium sulfate, 10 mM potassium chloride.

RNA Isolation Solution I – Phenol saturated with Tris buffer (pH 8.0) to a pH of 6.7 ± 0.2 was procured from Sigma, USA (catalogue number P4557). To this, SDS [0.1% (w/v)], NaOAc [0.32 M (w/v)] and EDTA (0.01 M, final concentration) from a stock solution of 0.5 M, pH 8.0) was added and mixed.

RNA loading dye (2X): Mix the following components in the concentrations given in the respective brackets and make up the final volume with ADW – 95 % formamide, 0.025 % SDS, 0.025 % bromophenol blue, 0.025 % xylene cyanol FF, 0.025 % ethidium bromide and 0.5 mM EDTA. Store the dye at – 20 °C.

SDS – PAGE reagents:

- **Acrylamide/Bis-acrylamide solution (30 %):** Dissolve 29.2 g of acrylamide and 0.8 g of bis-acrylamide in 50 ml of de-ionized water and make up the volume to 100 ml. Filter sterilize through 0.45 μm filter and store the solution in amber coloured bottle at 4 °C.
- **Tris – HCL, 1.5 M, pH 8.8 (100 ml):** Add 18.171 g Tris base (MW-121.14) in 60 ml of ADW and adjust the pH to 8.8 with HCL. Make up the volume to 100 ml with ADW and store at 4 °C
- **Tris – HCL, 0.5 M, pH 6.8 (100 ml):** Add 6.057 g Tris base (MW-121.14) in 60 ml of ADW and adjust the pH to 6.8 with HCL. Make up the volume to 100 ml with ADW and store at 4 °C
- **SDS (10 % w/v, 100 ml):** Dissolve 10 g SDS in 60 ml of ADW and mix by gentle stirring. Make up the volume to 100 ml with ADW
- **Ammonium Persulfate (10 % w/v, 1 ml):** Dissolve 100 mg ammonium persulfate in 1 ml ADW
- **Sample buffer, 5X:** 0.225 M Tris.Cl (pH 6.8), 50 % glycerol, 5 % SDS, 0.05 % bromophenol blue, 0.25 M DTT
- **Running buffer, 5X, pH 8.3:** To make 3 L of 5X running buffer, dissolve 45 g of Tris base, 216 g of glycine and 15 g of SDS in 2 L of ADW and make up the volume to 3 L. Do not adjust the pH with acid or base. Store at 4 °C. Warm to 37 °C before use if

precipitation occurs. 1X running buffer = 25 mM Tris, 192 mM gycine, 0.1 % SDS, pH 8.3.

- **Protein staining solution:** 50 % (v/v) Methanol, 10 % (v/v) Acetic acid, 0.1 % (w/v) Coomassie Brilliant Blue R250
- **Destaining solution:** 50 % (v/v) Methanol, 10 % (v/v) Acetic acid

SOC growth medium: The composition is as follows – tryptone (20 g/l), yeast extract (5 g/l), sodium chloride (0.5 g/l), magnesium sulfate ($MgSO4.7H_2O$, 5 g/l), glucose (3.60 g/l).

Sodium acetate (3 M) (MW=82.03): Dissolve 123.05 g of sodium acetate in 300 ml of DEPC treated water (unautoclaved) and adjust pH to 5.2 with the help of glacial acetic acid. Make up the final volume to 500 ml with DEPC treated water and autoclave.

TAE, 50X (1X = 40 mM Tris-acetate, 1 mM EDTA): Dissolve 121.05 g of Tris base in 400 ml of ADW. Add 28.55 ml of glacial acetic acid and 50 ml of 0.5 M EDTA, pH 8.0. Make up the volume to 500 ml and autoclave.

Tris-EDTA (TE, 1X) buffer, pH 8.0: 10mM Tris.Cl, 1 mM EDTA

Tris.Cl (1 M): Add 60.55 g of Tris base in 350 ml of DEPC treated autoclaved water. Adjust the pH to 8 using 10 N NaOH. Make up the final volume to 500 ml with DEPC treated water.

X gal (40 mg/ml): Dissolve 40 mg of X gal in 1 ml of DMSO and filter sterilize through 0.22 µm filter. Store in aliquots at -20 °C.

Appendix C: Primers and PCR parameters

Table 1 Degenerate primers and PCR conditions used for the amplification of ginsenoside biosynthetic genes

Gene	Primer sequence (5′ - 3′)	PCR conditions
acetyl-CoA C-acetyltransferase (*AACT*)	F: AGRGCAAAYGTBGAKCCR R: GCAGCWGCACCATCACTTAT	94 °C – 3 min; (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 40; 72 °C – 7 min
HMG-CoA synthase (*HMGS*)	F: ACAGYGCGGTCTATGCWG R: TGYTTGCCCWCBARTTTCTC	94 °C – 3 min; (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 38; 72 °C – 7 min Secondary PCR (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 38; 72 °C – 7 min
Mevalonate kinase (*MVK*)	F: GTTCATCWGCWGCWTTMT R: GAGCARMCCTTGRTTCAT	94 °C – 3 min; (94 °C – 30 s, 46 °C – 40 s, 72 °C – 1 min) x 35; 72 °C – 7 min Secondary PCR (94 °C – 30 s, 46 °C – 40 s, 72 °C – 1 min) x 38; 72 °C – 7 min
Phosphomevalonate kinase (*PMVK*)	F: ATGGCTGTWGTTGCTTCTGC R: CCATTAMAGGAAGTCATTKC	94 °C – 3 min; (94 °C – 30 s, 46 °C – 40 s, 72 °C – 1 min) x 35; 72 °C – 7 min Secondary PCR (94 °C – 30 s, 46 °C – 40 s, 72 °C – 1 min) x 40; 72 °C – 7 min
1-deoxy-D-xylulose-5-phosphate synthase (*DXS*)	F: GTYGGVATAGCDRARCARCRTG R: CATCWGAWGGAGCCATBACHAYC	94 °C – 3 min; (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 38; 72 °C – 7 min
1-deoxy-D-xylulose 5-phosphate reductoisomerase (*DXR*)	F: ARRGTTGTKGCWCTWGCWG R: GCAAGHGGMAGMACRAADGG	94 °C – 3 min; (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 38; 72 °C – 7 min Secondary PCR (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 40; 72 °C – 7 min
2-C-methyl-D-erythritol 4-phosphate	F: GTHTCDGTDRTTYTDYTDGCWGG	94 °C – 3 min; (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 38; 72 °C – 7

cytidylyltransferase (*CMS*)	R: CCTGDGGWGTYTGCATTTCCC	min <ins>Secondary PCR</ins> (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 40; 72 °C – 7 min
4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase (*CMK*)	F: GCDGGVYTHGGTGGTGGHAG R: CCRCTYCCRGACATRAAVAC	94 °C – 3 min; (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 35; 72 °C – 7 min <ins>Secondary PCR</ins> (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 40; 72 °C – 7 min
2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (*MCS*)	F: GAGGHTGYGARGCTCAYTCYGAT R: CCRAGRCTRTCDACYTTYTCA	94 °C – 3 min; (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 35; 72 °C – 7 min <ins>Secondary PCR</ins> (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 40; 72 °C – 7 min
4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (*HDS*)	F: GTCTKTCNGAYCGBATMATGAG R: GGRTCDATYTCYTCYTCTGG	94 °C – 3 min; (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 38; 72 °C – 7 min <ins>Secondary PCR</ins> (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 40; 72 °C – 7 min
4-hydroxy-3-methylbut-2-enyl diphosphate reductase (*HDR*)	F: ATTCAYAAYCCNACYGTBAA R: CCCTTHARCATHGTWGTTTG	94 °C – 3 min; (94 °C – 30 s, 45 °C – 40 s, 72 °C – 1 min) x 40; 72 °C – 7 min <ins>Secondary PCR</ins> (94 °C – 30 s, 45 °C – 40 s, 72 °C – 1 min) x 40; 72 °C – 7 min
isopentenyl diphosphate isomerase 2) (*IDI2*)	F: GYCATCCDCTVTACCGDG R: AMGGGGAYAGCTTCARACC	94 °C – 3 min; (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 38; 72 °C – 7 min <ins>Secondary PCR</ins> (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 40; 72 °C – 7 min
Squalene epoxidase (*SE*)	F: AGGGGTGCRATACAAAACMAAG R: TGTCCAGGCACATCAACYAAAC	94 °C – 3 min; (94 °C – 30 s, 54 °C – 40 s, 72 °C – 1 min) x 35; 72 °C – 7 min

Cycloartenol synthase (*CS*)	F: CAYTGTCGGATGGTKTAYCTGC R: CKRGTRTTYTCRTCYTCRTAATG	94 °C – 3 min; (94 °C – 30 s, 53 °C – 40 s, 72 °C – 1 min) x 35; 72 °C – 7 min Secondary PCR (94 °C – 30 s, 53 °C – 40 s, 72 °C – 1 min) x 35; 72 °C – 7 min
β – amyrin synthase (*β –AS*)	F: GACMTCTACTATCCYCATC R: CTTGCCATCCVTGATCTTG	94 °C – 3 min; (94 °C – 30 s, 50 °C – 40 s, 72 °C – 1 min) x 38; 72 °C – 7 min
protopanaxadiol synthase (*PPDS*)	F: CAACGCAGACAACTTCTCC R: GGCACGATTCATAGCAGTC	94 °C – 3 min; (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 38; 72 °C – 7 min Secondary PCR (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 40; 72 °C – 7 min
protopanaxatriol synthase (*PPTS*)	F: GCTGACTTTTGAGTTGGCC R: GCRATCTCCATTTGTTCTGTG	94 °C – 3 min; (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 38; 72 °C – 7 min Secondary PCR (94 °C – 30 s, 52 °C – 40 s, 72 °C – 1 min) x 40; 72 °C – 7 min

F – forward primer, R – reverse primer; R – A/G, Y – C/T, S – C/G, W – A/T, K – G/T, M – A/C, B – C/G/T, D – A/G/T, H – A/C/T, V – A/C/G, N – A/C/G/T

Table 2 RACE primers

Gene	Primer name	Primer sequence (5′ - 3′)	No. of cycles
Farnesyl pyrophosphate synthase	FPS_PF	GGGAACCTATTTCAAGTGCAGGATGA	30
	FPS_NF	GGGGAAGATTGGCACAGATATTGAAGA	30
	FPS_PR	TAAGGCTTTTGTCGGAAATGCTTCTTG	30
	FPS_NR	TCTTGAGAATCCTTGGGATATGGTTGC	30
Squalene synthase	SS_PF	TGATAAGGACTGGCACTTTTCATGTGG	30
	SS_NF	GGCCTCGCCAGATTTGGAGTAA	30
	SS_PR	CGCTTCCAAGCTCAAGAAAAGCATTAG	30
	SS_NR	ACAGTGTCAAGTGCTCGAAGAACCAAA	30

Squalene epoxidase	SE_PF	TGGTTGTTTTTCGAATCTTCGGCATTC	30
	SE_NF	GTTTTTCGAATCTTCGGCATTCCCTCT	30
	SE_PR	CTTTGTCCAGGCACATCAACTAAACAG	30
	SE_NR	ACATCAACTAAACAGCGAATTTCGGTG	30
Dammarenediol synthase	DMS_PF	CCTTCAAGTTTTCCTTTTCATCCAGCA	30
	DMS_NF	GGTGCACTTACATGCCAATGTCGTATT	30
	DMS_PR	TGCTGGATGAAAAGGAAAACTTGAAGG	30
	DMS_NR	AGGAAAACTTGAAGGGAAAAGCCAGAA	30
Cycloartenol synthase	CS_PF	TGCTCTCTTTGAGAAAGGGGCTCTTCA	30
	CS_NF	GGGCTCTTCACTGTCCCTTATCACGAA	30
	CS_PR	CGAGTATTCTCGTCTTCATAATGAATG	30
	CS_NR	GCCAACGCATGAAAATAGGTTCCAAGA	30
CMK (4-diphosphocytidyl-2-C-methyl-D-erythritol kinase)	CMK_PF	CAAGCTATTTCTATTGACCCTCTGACC	30
	CMK_NF	CCTGCATTCGAAGTCCTCCCATC	30
	CMK_PR	CCTGTACAATATGCTGCTCCGTGAG	30
	CMK_NR	CATTCTTGCAGTTCCTTTTCAGTGGCA	30
Phosphomevalonate kinase	PMVK_PF	TCAAGTCACTCAAGGAACACCTTTGTG	30
	PMVK_NF	GGCATATGCAAGCCTCGACAATGATAA	30
	PMVK_PR	GTCCATTCCGAAGCCAAGCTGTC	30
	PMVK_NR	GTCCATTCCGAAGCCAAGCTGTC*	30

#PCR parameters were same for all the genes: 94 °C – 10 s, 68 °C – 30 s, 72 °C – 3 min. * In case of 5′ RACE, same primer was used in primary and nested PCR; PF: Primary forward (3′ primary), NF: Nested forward (3′ nested), PR: Primary reverse (5′ primary), NR: Nested reverse (5′ nested)

Table 3 Primers for semi – qRT PCR

Gene name	Primers (5′ - 3′)	*No. of cycles
Galactinol synthase 2 (JZ822902)	F: GCAAAGGGTTTAAGAAAGGT R: CAATCTCATGAATGATGCAC	30
Cell division cycle 20.1, cofactor of APC complex-like (JZ822903)	F: ATGATAACGGAGCTTTATGG R: TCCCAAGAATGTTCCATATT	40
Metallothionein like protein type 3(JZ822923)	F: CGTCTCAACATAGCTCTTCC R: TTGAATTACTCACCATGTCG	28
GBR5-like protein (JZ822900)	F: CTGCCATTATAGGTGCTGTA R: CATGCATCGTCAGATCAA	30

PsbA (JZ822896)	F: TACCTTATTGACCGCAACTT R: GTAGGAATAATGGCACCTGA	30
Protein KIAA0664 homolog (JZ822767)	F: CTCTCTCCATTGAAGCAGTC R: TAGCTGTGAGAGTGGAAGGT	35
Major latex-like protein (JZ822739)	F: AGGTGATTGAAGGACATTTG R: GAGCTTCTCATAGTCGATGG	30
Rnase-like major storage protein (JZ822836)	F: ACTCGGAATATGTGCTTACG R: GAGGCGGTAGTCATCTGTAG	30
Glyceraldehyde-3-phosphate dehydrogenase (JZ822833)	F: GTTCTCATTCAAAGCAATCC R: GATGTTGTATCCACGGACTT	30
Ankyrin repeat-containing protein (JZ822856)	F: TGAGTTGAGGAACACTGTGA R: GTTTCCTGAGCTCTTTAGCA	35
β – amylase (JZ822864)	F: GGGAGCTCAGATATCCTTCT R: TGGATCCAAAGAACTCTGTC	30
Transcription factor bHLH96-like (JZ822738)	F: GTACTCCGGTGGTCAGTTAC R: CCTTGTTCTTGGAACTTCTG	40
Ubiquitin-conjugating enzyme E2 (JZ822811)	F: CAACAGCGAACAGATAGACA R: AGCATTTGCCTTGATATTCT	30
Heat shock protein 70 (JZ822879)	F: TCCTCTTAAATTCCTGGACA R: TTGATGTGTCCCTTCTTACC	35
Polyubiquitin like protein (JZ822849)	F: TTGTAGTCAGCCAAAGTCCT R: CAAAGATCCAGGATAAGGAG	30
Farnesyl diphosphate synthase (KT936527)	F: TTTCTTGTGCTCGATGATATT R: AGAATCCTTGGGATATGGTT	38
Squalene synthase (KT936528)	F: TACCAGGAGGCAATAGAAGA R: CCTAATCCAACAAGTTCTGC	35
Dammarenediol synthase (KU196775)	F: CCTCTTGGGGAAAGACTTAT R: TGAAAAGGAAAACTTGAAGG	27
18S rRNA	F: ACACGGGGAGGTAGTGA	30

	R: CTCCAATGGATCCTCGTTA	

*PCR parameters were same for all genes: 94 °C – 3 min, (94 °C – 30 s, 58 °C – 40 s, 72 °C – 1 min), 72 °C – 7 min

Table 4 Primers for qRT PCR

Gene name	Primers (5′ - 3′)
Farnesyl diphosphate synthase (KT936527)	F: TTTCTTGTGCTCGATGATATT R: AGAATCCTTGGGATATGGTT
Squalene synthase (KT936528)	F: TACCAGGAGGCAATAGAAGA R: CCTAATCCAACAAGTTCTGC
Dammarenediol synthase (KU196775)	F: CCTCTTGGGGAAAGACTTAT R: TGAAAAGGAAAACTTGAAGG
Squalene expoxidase (KT936529)	F: AACAGCGAATCTCGGTACTA R: CTGACAATTGTTTGTGATGG
Cycloartenol synthase (KT936530)	F: AACCTATGTGCAAAGGAAGA R: ATCACAGTGCGAAGAGACTT
18S rRNA	F: ACACGGGGAGGTAGTGA R: CTCCAATGGATCCTCGTTA

PCR parameters were same for all genes: 94 °C – 10 min, (94 °C – 30 s, 58 °C – 30 s, 72 °C – 30 s) x 40 cycles

Table 5 Primers used to prepare construct for heterologous expression of FPS

Primer	Sequence (5′ – 3′)	Purpose
FPS_ORF_Forward	ATGAGCGATTTGAAGAC	Amplification of ORF, forward primer
FPS_ORF_Reverse	TTACTTTTGCCGCTTATA	Amplification of ORF, reverse primer
FPS_ectopic_forward	*GCATGCATGAGCGATTTGAAGA	Introduction of cloning sites, forward primer
99FPS_ectopic_reverse	#CTGCAGTTACTTTTGCCGCTTAT	Introduction of cloning sites, reverse primer

*Restriction sequence for *Pae*I; #Restriction sequence for *Pst*I. PCR parameters are mentioned in "Chapter 3: Materials and methods".

Table 6 Other primers used in the study

Primer	Sequence (5′ – 3′)	Purpose
M_{13} forward	GTAAAACGACGGCCAGTG	Colony PCR to check insert cloned in pCR@2.1 vector
M_{13} reverse	GGAAACAGCTATGACCATG	
Univeral Primer A mix (UPM)	Long: CTAATACGACTCACTATAGGGCA AGCAGTGGTATCAACGCAGAGT Short: CTAATACGACTCACTATAGGGC	Primary RACE PCR
Nested Universal Primer A (NUP)	AAGCAGTGGTATCAACGCAGAGT	Nested RACE PCR
pQE forward	CCCGAAAAGTGCCACCTG	Sequencing primers to check the insert in pQE30 vector
pQE reverse	GTTCTGAGGTCATTACTGG	

Appendix D: List of thesis related publications

1. Gurung B, Bhardwaj PK, Talukdar NC. 2016. Subtractive transcriptome analysis of leaf and rhizome reveals differentially expressed transcripts in *Panax sokpayensis*. Funct Integr Genomics, 16, 619–639.
2. Gurung B, Bhardwaj PK, Rai AK, Sahoo D. 2018. Major ginsenoside contents in rhizomes of *Panax sokpayensis* and *Panax bipinnatifidus*. Nat Prod Res, 32, 234–238.
3. Gurung B, Saha D, Bhardwaj PK, Sahoo D. 2018. Cloning and *in silico* characterization of *phosphomevalonate kinase* gene from *Panax sokpayensis* Shiva K Sharma & Pandit. (Accepted: Environment & Ecology, *ISSN 0970-0420*).