# Chapter 1: Introduction

Ginsenosides are triterpene glycoside saponins, produced by different species of genus *Panax* L. (Araliaceae) which is commonly known as ginseng. *Panax* species that are commercially cultivated and extensively traded are *Panax notoginseng* (Burk) F. H. Chen (Chinese ginseng), *Panax ginseng* Meyer (Korean ginseng) and *Panax quinquefolius* L. (American ginseng) (Baeg and So, 2013). The global market of ginseng (roots plus processed goods) was estimated at $2085 million with a total root production of 80,080 tons as on 2009 (Baeg and So, 2013). The plant roots are used as health tonic and are incorporated into a variety of commercial health products like ginseng soups, drinks, capsules and cosmetics in the Asian as well as international market (Paek et al., 2005). Ginseng has been used as traditional herbal medicine and food for over 2000 years (Attele et al., 1999; Kim, 2012; Liang and Zhao, 2008; Xiang et al., 2008). Ginsenosides have cardioprotective, immunomodulatory, antifatigue, anticancerous, antidiabetic and antioxidant properties (Christensen, 2009; Lee and Kim, 2014; Sodrul et al., 2018; Xiao et al., 2015).

## 1.1. *Panax sokpayensis* Shiva K. Sharma & Pandit

A new species of *Panax* christened as *Panax sokpayensis* Shiva K. Sharma & Pandit was reported from Sikkim Himalaya, India in 2009 (Sharma and Pandit, 2009). According to the latest classification, only two species namely *P. sokpayensis* and *P. sikkimensis/ P. bipinnatifidus* are found in Sikkim Himalaya (Sharma and Pandit, 2009, 2011). *P. sokpayensis* grows at an altitude of 1700 – 2300 m above sea level in the submontane terrain of Uttarey, Sopakha and Yuksom in West Sikkim (Figure 1.1). It is a robust perennial herb with horizontal rhizome with distinct stem scars and rings at nodes, short thick internodes and at the end of the rhizome, a single globose tuber is present (Figure 1.2). The stem is cylindrical, erect and is deciduous. The stem bears 4-5 petiolate, palmately compound leaves. Each leaf in turn contains 5 leaflets (Figure 1.2). Fruits are subglobose and when they are ripe, the upper half portion is black and lower portion is red in colour (Figure 1.2). The plant is named after the village, “Sopakha” (altitude 2200 m; N27° 16′ 17″, E88° 04′ 55″), West Sikkim. Owing to high medicinal value of the species, the rhizomes are harvested for both domestic use and trade. Ginsenosides present in *P. sokpayensis* are the primary source of significant medicinal properties and the quality and quantity of these

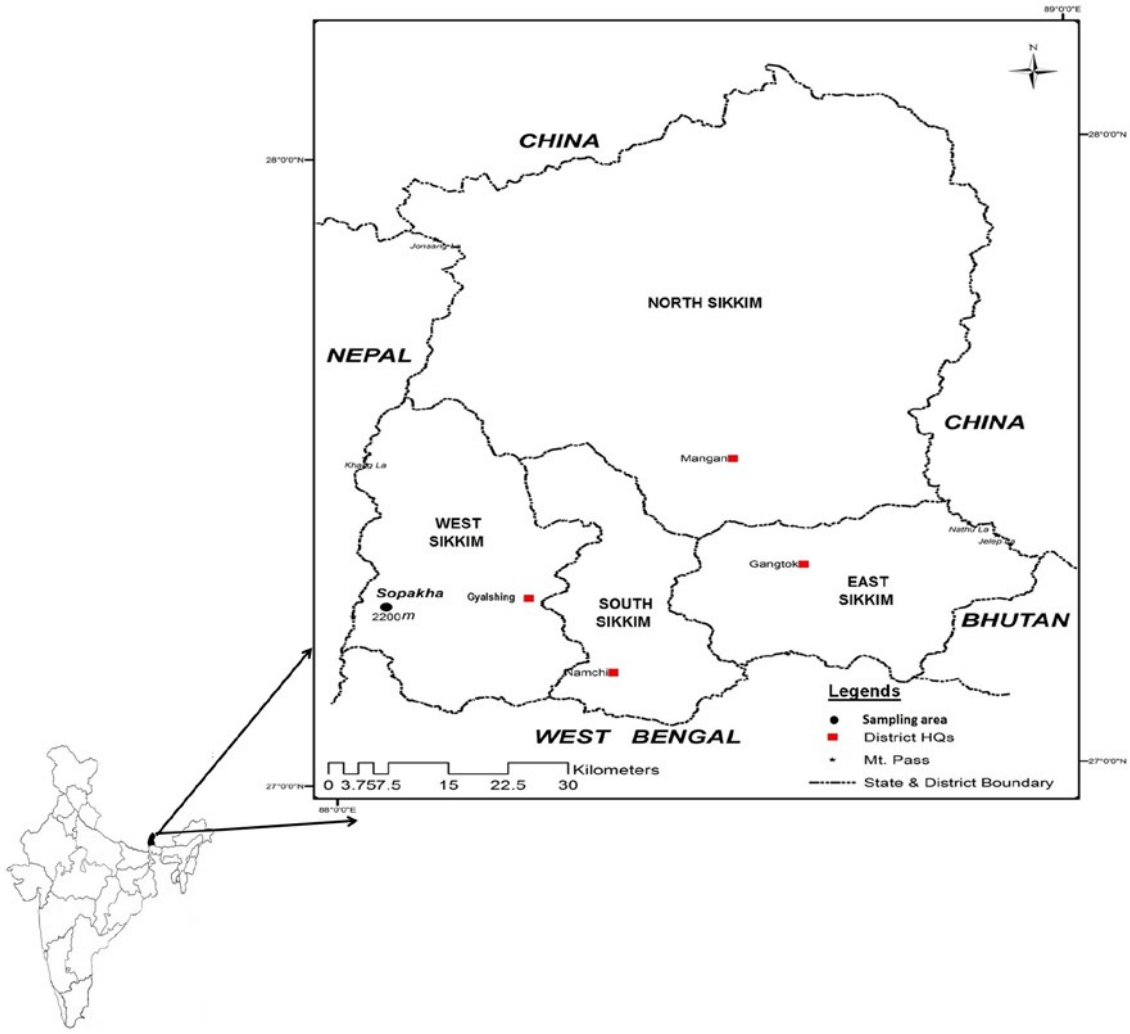


**Figure 1.1** Map depicting the location of *P. sokpayensis* in Sikkim (India).

active ingredients in this species are at par with those of an Asian ginseng, viz., *P. ginseng* (Gurung et al., 2018). These ginsenosides are synthesized through ginsenoside biosynthetic biosynthetic pathway which derives its precursors from mevalonate and non-mevalonate pathways as reported in various *Panax* species (Kim et al., 2009c ; Li et al., 2013; Luo et al., 2011; Sun et al., 2010a).

## 1.2. Biosynthesis of ginsenosides

The mevalonate/mevalonic acid (MVA) and methylerythritol phosphate (MEP) pathways channelize the carbon flux from the primary metabolism to the ginsenoside biosynthetic pathway (Figure 1.4A). Both MVA and MEP pathways produce 5– carbon (C5) isomers isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) as end


**Figure 1.2** *P. sokpayensis* growing in the wild habitat at Sopakha forest, West Sikkim, India. (A, B, C) Exploration of natural habitats of *P. sokpayensis* (D) *P. sokpayensis* without flower and fruits (E) *P. sokpayensis* with fruits (F) Rhizome of *P. sokpayensis*.

products that act as precursors for the ginsenoside biosynthetic pathway (Wang et al., 2012; Zhao et al., 2014).

In the classical cytosolic MVA pathway, three molecules of acetyl-CoA from primary glucose metabolism are condensed successively to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) (Concepcion and Boronat, 2015; Miziorko, 2011). The first such reaction is catalyzed by acetoacetyl-CoA thiolase (AACT) to form acetoacetyl-CoA. HMG-CoA synthase (HMGS) carries out the second condensation reaction to form HMG-CoA which is reduced by HMG-CoA reductase (HMGR) to form MVA. MVA is then phosphorylated by mevalonate kinase (MVK) to give mevalonate 5 – phosphate (MVP). This intermediate is further phosphorylated by phosphomevalonaote kinase (PMVK) to produce mevalonate 5-diphosphate (MVPP) which undergoes decarboxylation catalyzed by mevalonate diphosphate decarboxylase (MVD) to form IPP.

The plastidial MEP pathway (Figure 1.4A) starts with the condensation of D-glyceraldehyde 3-phosphate (GAP) and pyruvate to form 1-deoxy-D-xylulose 5-phosphate (DXP) in a reaction catalyzed by DXP synthase (DXS) (Dubey et al., 2003; Eisenreich et al., 2004; Kuzuyama and Seto, 2012). DXP is reduced by DXP reductoisomerase (DXR) to 2-*C*- methyl-D-erythritol 4-phosphate (MEP) which is converted to 4- (diphosphocytidyl) -2-C-methyl-D-erythritol (CDP-ME) by MEP cytidylyltransferase (CMS). CDP-ME kinase (CMK) acts on CDP-ME to catalyze the formation of 4-(diphosphocytidyl)-2-C- methyl- D-erythritol-2- phosphate (CDP-MEP) which is converted to cyclic intermediate 2-C-methyl-D-erythritol 2,4 cyclodiphosphate (MEcPP) catalyzed by MEcPP synthase (MCS). MEcPP is then converted to (E)-4-hydroxy-3-methylbut-2-en-1-yl diphosphate (HMBDP) by HMBDP synthase (HDS). In the final step, HMBDP reductase (HDR) reduces HMBDP to IPP and DMAPP. The IPP produced through both MVA and MEP pathways undergoes isomerization under the action of isopentenyl diphosphate isomerise (IDI).

Different ginsenosides are biosynthesized with the sequential 1′–4 condensation of IPP with DMAPP and then with the resultant geranyl pyrophosphate (GPP) to give farnesyl pyrophosphate (FPP) catalyzed by geranyl pyrophosphate synthase (GPS) and farnesyl pyrophosphate synthase (FPS), respectively (Gurung et al., 2016). Two FPP molecules are then combined by squalene synthase (SS) to give squalene. In the next step, squalene epoxidase (SE) catalyzes production of 2, 3–oxidosqualene from squalene. Different types of oxidosqualene cyclases cyclize 2, 3–oxidosqualene to form intermediates for different types of triterpenes (Kim et al., 2009c; Wang et al., 2012): cycloartenol synthase (CS) cyclizes 2, 3–oxidosqualene to cycloartenol which leads to the formation of sterols while

lupeol synhtase (LS) cyclizes it to form lupeol. β – amyrin synthase (β – AS) converts 2, 3–oxidosqualene to β – amyrin which is an intermediate in the biosynthesis of oleanane type ginsenoside, Ro (Figure 1.3C) while dammarenediol synthase (DS) catalyzes 2, 3–oxidosqualene to produce dammarenediol, an intermediate for all dammarane type ginsenosides. A Cytochrome P450 enzyme (CYP) namely dammarenediol 12 – hydroxylase (protopanaxadiol synthase, PPDS) hydroxylates dammarenediol to protopanaxadiol (Han et al., 2011) which in turn is further hydroxylated at C – 6 position by another CYP, protopanaxatriol synthase (PPTS) to form protopanaxatriol (Han et al., 2012). Glycosylation of protopanaxadiol and protopanaxatriol by different glycosyltransferases (GTs) leads to the production of different protopanaxadiol type (Ra1, Ra2, Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2, etc.) and protopanaxatriol type (Re, Rf, Rg1, Rg2, Rh1, etc.) ginsenosides, respectively (Figure 1.3A, B).

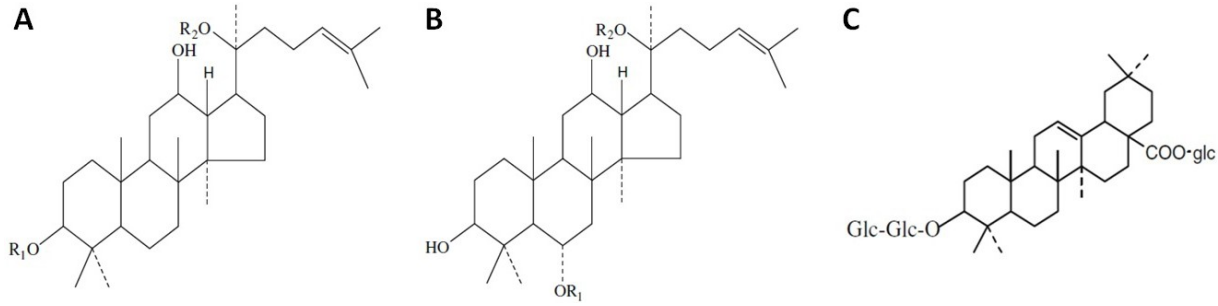


**Figure 1.3** Chemical structures of ginsenosides. (A) Protopanaxadiol (B) Protopanaxatriol (C) Ginsenoside Ro (Oleanane type of ginsenoside). Glc – glucopyranoside; $R_1$, $R_2$ – molecules or atoms that attaches to the main skeleton. Source: Wang et al., 2012.

## 1.3. Global status of ginseng research

Ginsenoside profiles, their pharmacological activities and biosynthetic pathways have been studied in different *Panax* species. Some of the biosynthetic pathway genes have been cloned and characterized (Kim et al., 2009c; Liang and Zhao, 2008; Wang et al., 2012). Recently, genes from ginsenoside biosynthetic pathway have been studied using *de novo* sequencing and transcriptome studies in *P. notoginseng* (Liu et al., 2015; Luo et al., 2011), *P. quinquefolius* (Sun et al., 2010a; Wang et al., 2016b; Wu et al., 2013), *P. ginseng* (Jayakodi et al., 2014; Li et al., 2013; Subramaniyam et al., 2014; Zhang et al., 2017), *P. vietnamensis* var *fuscidiscus* (Zhang et al., 2015a) and *P. japonicus* (Rai et al., 2016). *P. sokpayensis* is distributed on altitudes ranging from 1900 m to 2300 m above mean sea level in the West district of Sikkim (Sharma and Pandit, 2009). It is a shade loving plant. Owing

to its humid and shady habitat, the plants are frequently exposed to different pathogens and abiotic stresses. Intriguingly, some *P. sokpayensis* plants were also found growing for more than 20 years under

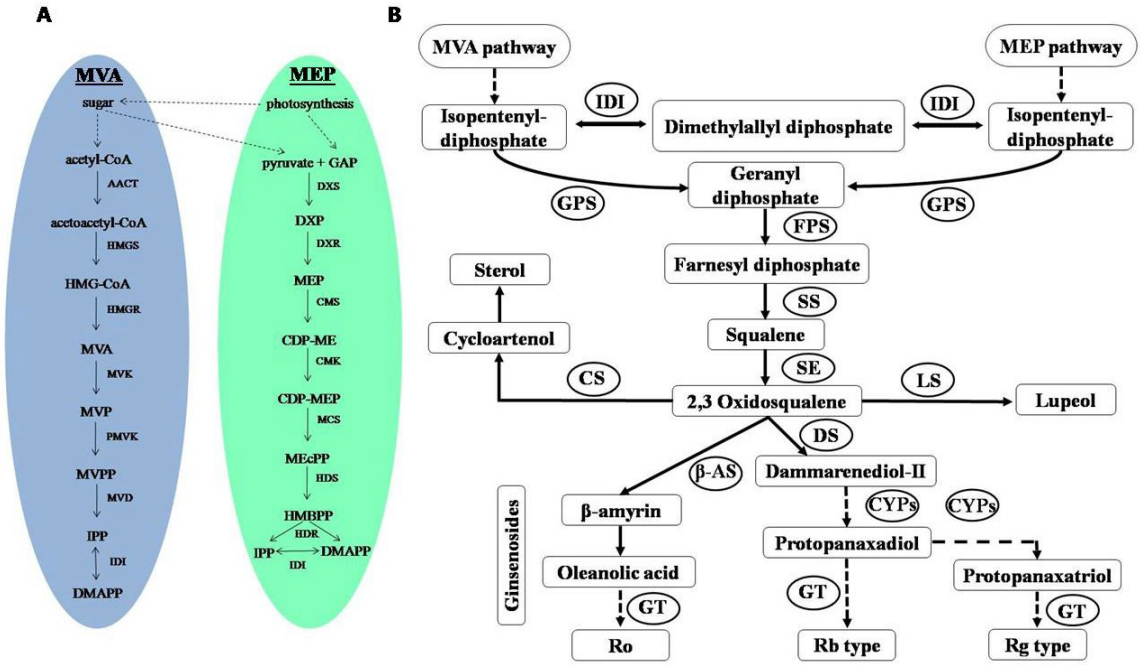


**Figure 1.4** Proposed ginsenoside biosynthetic pathway as adapted from Gurung et al., 2016. (A) Mevalonate (MVA) and methylerythritol phosphate (MEP) pathways (B) Ginsenoside biosynthetic pathway. AACT: Acetoacetyl-CoA thiolase, HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA, HMGS: HMG-CoA synthase, HMGR: HMG-CoA reductase, MVA: Mevalonate, MVK: Mevalonate kinase, MVP: Mevalonate 5– phosphate, PMVK: Phosphomevalonate kinase, MVPP: Mevalonate 5-diphosphate, MVD: Mevalonate diphosphate decarboxylase, IPP: Isopentenyl 5-diphosphate, DMAPP: Dimethylallyl diphosphate; GAP: D-glyceraldehyde 3-phosphate, DXP: 1-deoxy-D-xylulose 5-phosphate, DXS: DXP synthase, DXR: DXP reductoisomerase, MEP: 2-*C*- methyl-D-erythritol 4-phosphate, CMS: MEP cytidylyltransferase, CDP-ME: 4- (diphosphocytidyl) -2-C-methyl-D-erythritol, CMK: CDP-ME kinase, CDP-MEP: 4-(diphosphocytidyl)-2-C- methyl- D-erythritol-2- phosphate, MEcPP: 2-C-methyl-D-erythritol 2,4 cyclodiphosphate, MCS: MEcPP synthase, HMBDP: (E)-4-hydroxy-3-methylbut-2-en-1-yl diphosphate, HDS: HMBDP synthase, HDR: HMBDP reductase. IDI: Isopentenyl diphosphate isomerase, GPS: Geranyl pyrophosphate synthase, FPS: Farnesyl pyrophosphate synthase, SS: Squalene synthase, SE: Squalene epoxidase, CS: Cycloartenol synthase, LS: Lupeol synthase, β-AS- β-amyrin synthase, DS: Dammarenediol synthase, CYP: Cytochrome P450, GT: Glycosyltransferase.

high-humid, low-temperature, and rainy conditions in the niche habitat. Ginsenosides present in *P. sokpayensis* might play key roles in the adaptation of plants to its niche environment. As suggested by their bitter tastes, they may also have roles in plant defense against pathogens and insects. Molecular information on the biosynthesis of ginsenosides, an important group of triterpene secondary metabolites is lacking in *P. sokpayensis*.

Therefore, following objectives are proposed for the present research:

1. Identification and cloning of genes involved in the biosynthesis of ginsenosides from *P. sokpayensis*.
2. Characterization of genes involved in the biosynthesis of ginsenosides.
3. Developing appropriate expression system for identified gene (s).