In Vitro Production of Saponins

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Abstract

Plants have been utilized as food, feed, and fodder since the dawn of civilization. Plants are also thought to be a rich source of bioactive compounds with a variety of pharmacological actions. Saponins are one such group of molecules which are present in various plant species. As triterpenoid glycosides, they have a 30C oxidosqualene precursor aglycone moiety (sapogenin), which is then linked with glycosyl residues to form saponin. These saponins have a unique platform in the field of pharmaceutical and nutraceutical industries. Saponins are used for the treatment of various diseases which include cancer, diabetic, cardiac, hepatic, and nervous disorders. The production of saponins through conventional approaches is time-consuming and hard to extract pure compounds, and thus to achieve this, in vitro methods have been developed and enhanced the production and extraction of the metabolites. The present chapter focuses on the in vitro production of saponins through various tissue culture techniques such as shoot, callus, cell suspension, adventitious root, hairy root culture, and applications of bioreactors at commercial level. The chapter also focuses on biosynthetic pathway, extraction methods, and biological activities of saponins.

Keywords

Saponins · Plant secondary metabolite · In vitro production · Biosynthesis · Pharmacology

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10.1 Introduction

Saponins are plant secondary metabolites derived from mevalonic acid pathway which are amphipathic glycosides of triterpenes and steroids, often also called as steroidal glycoalkaloids. 'Sapo' in Latin refers to soap as these compounds produce foams when shaken with aqueous solutions (Mugford and Osbourn 2012). Saponins are large molecules containing a hydrophilic sugar moiety at one end separated from hydrophobic (lipophilic) non-sugar triterpene or steroid moiety. This property enables them to form a micelle and act as a detergent (Mishra et al. 2017). The non-sugar component is called aglycone (sapogenin) and composed of triterpenoid or steroidal backbone and the sugar component is called glycone and composed of molecules like arabinose, xylose, glucose, galactose, fructose, rhamnose, and glucuronic acid (Moghimipour and Handali 2015) Fig. 10.1. Based on their molecular and chemical nature, they are divided into triterpenoid (30 carbon atoms) and steroidal saponins (27 carbon atoms with 6-ringed spirostane or 5-ringed furostane skeleton). Dammaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasteranes, ursanes, cycloartanes, lanostanes, cucurbitanes, and steroids are among the 11 saponin classes. In the plant kingdom, oleananes are found more often (Kregiel et al. 2017) Fig. 10.2.

Triterpenoid saponins play a vital role in plant physiology during the external stress conditions. Apart from these due to their pharmacological properties, they are extensively used in various fields of medicine (Yao et al. 2020). The antiviral properties of saponins inhibit the replication of Herpes simplex virus type-1 and

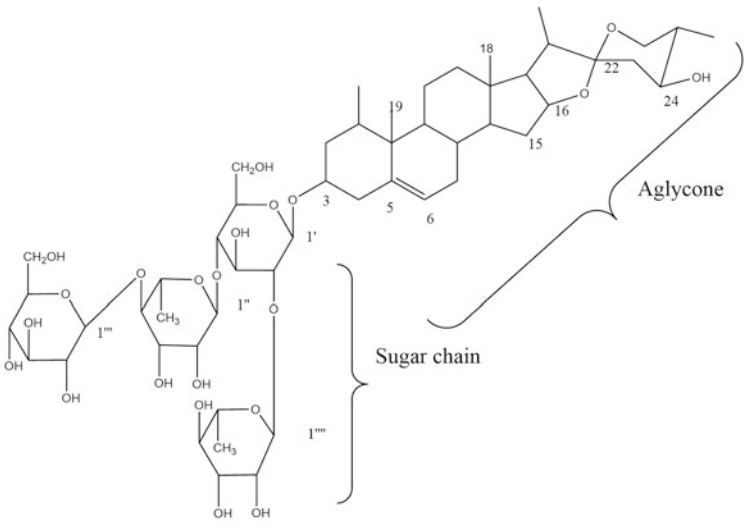

Fig. 10.1 Structure of Saponin. (Source: Moghimipour and Handali 2015)

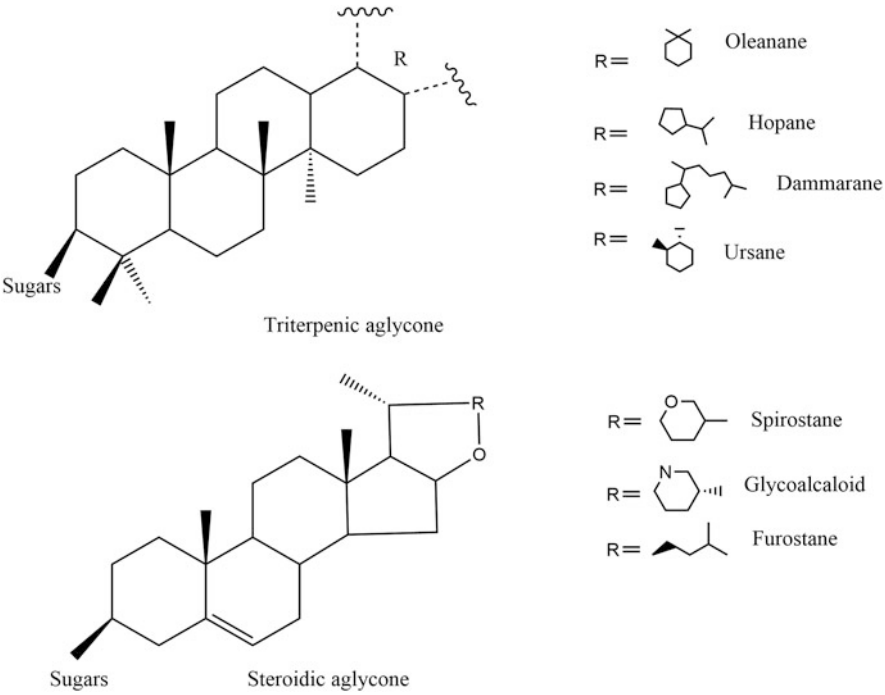

Fig. 10.2 Structure of triterpenoid and steroidal saponins. (Source: Moghimipour and Handali 2015)

Polio virus type-2 (Amoros et al. 1987). Plants extracts with saponins are used as animal feed for dairy and beef cattle as they inhibit rumen ciliate protozoans (Holtshausen et al. 2009), also suppress methane emission and change the fermentation patterns (Hu et al. 2005). Saponins in plants act as 'phytoprotectants' or 'phytoanticipins' as they act as a defence molecule against microbes and pest attack. They also play an important role in cell membrane permeability. Apart from these, they possess anti-inflammatory, anti-microbial, hyperlipidemic, and hypoglycemic activity (Desai et al. 2009). The triterpenoids play a major role in preventive and curative healthcare, perfume industries, cosmetics, flavouring, food, and beverage industries. (Biswas and Dwivedi 2019).

Depending on its growth phase and development, as well as seasonal variations, various plant species synthesize and accumulate varying quantities of saponins in different plant regions (Table 10.1). Saponin production may also be stimulated in response to external biotic stress, such as herbivores and disease attacks. Abiotic stresses such as light, temperature, and nutritional deficiency can all have an impact on both the quality and amount of saponin content. It has been revealed that at the molecular level, their synthesis is assisted by the transcriptional activation of their respective biosynthetic genes via a complicated signalling cascade including the

Table 10.1 Saponin content of some selected plant materials

Sl. No.	Name of the plant	Source	Saponin content	Reference
1	*Chenopodium quinoa* Willd	Seed	7.51–12.12 mg OAE/g	Han et al. (2019)
2	*Sapindus mukorossi* Gaertn.	Pericarp	280.55 ± 6.81 mg/g	Deng et al. (2019)
3	*Aloe vera* (L.) Burm. f.	Leaves	65.89 mg OAE/g	Akbari et al. (2021)
4	*Aesculus hippocastanum* L.	Seed	3–6%	Güçlü-Ustündağ and Mazza 2007)
5	*Primula grandis* L.	Roots	15–20%	Włodarczyk et al. (2020)
6	*Glycyrrhiza glabra* L.	Roots	3.6 g/200 g	Hajimohammadi et al. (2017)
7	*Yucca schidigera* Roezl.	Truck	10	Oleszek et al. (2001)
8	*Trigonella foenum-graecum* L.	Seed	0.98%	Chaudhary et al. (2018)
9	*Panax notoginseng* (Burkill) F.H.Chen	Root	9.26–46.52 mg/g	Cui et al. (2019)
10	*Glycine max* (L.) Merr.	Seed	1173.5 to 3582.3 mg/100 g	Lee et al. (2020)
11	*Avena sativa* L.	Seed bran	4.6%	Ralla et al. (2018)
12	*Medicago sativa* L.	Aerial parts	6.5–9.5 mg/g DW	Zhang et al. (2021)
13	*Bacopa monnieri* (L.) Pennell	Leaves	13–38.12 mg/g DW	Bhardwaj et al. (2019)
14	*Centella asiatica* (L.) Urban	Leaves	1.2–2 mg/g DW	Mangas et al. (2008)
15	*Gymnema sylvestre* R. Br.	Leaves	397.9 mg/g DW	Sheoran et al. (2015)
16	*Ziziphus joazeiro*	Bark	2–10%	Ribeiro et al. (2014)

jasmonate and salicylate hormones. Exogenous effect of stress-causative factors (elicitors) on secondary metabolite synthesis is frequently used to upregulate production of these essential bioactive metabolites (Lambert et al. 2011; Biswas and Dwivedi 2019).

However, the synthesis of this therapeutically significant class of bioactive compounds in the plant system in vivo is exceedingly low, leading to massive overuse of wild plant populations for their procurement by the pharmaceutical companies. Furthermore, with little or no planned re-cultivation, these techniques frequently endanger the plant populations. Plant tissue culture techniques like cell and organ cultures serve as an alternative for sustained and quality synthesis of these metabolites. The possibility of scaling up these cultures to the commercial level contributes to the industrial potential of metabolite synthesis using tissue cultures (Namdeo 2007; Biswas and Dwivedi 2019).

The present chapter deals with the production of saponins from cell and organ cultures and engineering strategies for enhanced metabolite content. Also, emphasis has been given for the biosynthesis of saponins and the key genes involved in the biosynthetic pathway have been mentioned. Furthermore, the different extraction and quantification methods employed for saponins and their potential biological activities have been discussed.

10.2 Biosynthesis of Saponins

Saponins are broadly classified as steroidal saponins or triterpenoid saponins based on the type of aglycone backbone from which it is derived. While triterpenoid saponins are synthesized majorly by dicotyledonous plants and are the most abundant class of saponins, steroidal saponins are mainly synthesized by monocotyledonous plants. Both triterpenoid and steroidal saponins are synthesized from the same oxidosqualene precursor which is 30 carbon long and linear. While the triterpenoid aglycone backbone contains all 30 carbons of oxidosqualene, the steroidal aglycone backbone retains only 27 carbons and loses 3 methyl groups. If additional nitrogen is incorporated to the steroidal aglycone backbone, it functions as a precursor for the biosynthesis of steroidal glycoalkaloids which may also be sometimes considered as saponins (Augustin et al. 2011; Friedman 2006; Ginzberg et al. 2009; Itkin et al. 2013).

The steroidal aglycone and triterpenoid aglycone being isoprenoids are synthesized using Isopentenyl pyrophosphate (IPP) as precursors that are converted from Acetyl CoA through the Mevalonate (MVA) pathway which is also known as 3-Hydroxy-3-methylglutaryl-CoA-reductase (HMGR) pathway. Further, Isopentenyl diphosphate isomerase (IDI) isomerizes IPP to allylic form dimethylallyl pyrophosphate (DMAPP). One molecule of the 5 carbon DMAPP then condenses with two molecules of 5 carbon IPP to give a 15-carbon immediate prenylated precursor of saponins, called Farnesyl pyrophosphate (FPP). Squalene synthase is a key enzyme in saponin biosynthesis. It catalyses the formation of a 30-carbon precursor, squalene, by the condensation of two molecules of FPP. Squalene is then epoxidized by the action of squalene epoxidase to form 2,3-oxidosqualene. A variety of cyclizing enzymes of the class oxidosqualene cyclase carry out the cyclization of 2,3-Oxidosqualene to form polycyclic structures. This reaction is the branching point between the metabolism of primary and specialized triterpene in higher plants.

The 2,3-Oxidosqualene cyclization results in one of the earliest inherent diversities to the triterpenoid saponins aglycones, accounting for its tendency to give rise to a vast array of triterpenoid scaffolds arising from a single substrate due to several carbocation rearrangements in the cyclization process. Majorly, there are 9 classes of triterpene backbones in plants synthesized by either specific or multifunctional Oxidosqualene cyclase to produce either single or multiple products through a single cyclization reaction of 2,3-Oxidosqualene (Vincken et al. 2007). One such cyclized structure, namely Cycloartenol, is a tetracyclic precursor of

primary terpene formed by 2,3-Oxidosqualine cyclization catalysed by Cycloartenol synthase. Cycloartenol is a precursor for numerous phytosterols in angiosperms including carbon sitosterol, while all other cyclization products get involved as precursors for specialized triterpenes synthesis. The cholesterol backbone of these Cycloartenol derivatives undergoes several glycosylations and oxygenations to give rise to Spirostanol or Furostanol derivatives containing an oxygen heteroatom in their aglycone structure that further forms steroidal saponins (Thakur et al. 2011). Aglycones such as Solanidine, Solasodine, Tomatidine, and Demissidine are formed by cholesterol precursors that are utilized by steroidal glycoalkaloids, where an amine group is incorporated as a heteroatom instead of oxygen, through a series of side-chain modifications (Itkin et al. 2013; Ginzberg et al. 2009).

These triterpene aglycones are, although, majorly oxidized by multiple Cytochrome P450-dependent monooxygenases (P450s), several other modifications are also carried out that contribute to the extended diversity in the structure of the aglycone backbone by adding a second level of complexity. Various transferases including UDP-dependent glycosyltransferases (UGTs) and acyltransferases catalyses the modification of reactive functional groups and normalize the polarity of scaffolds that were introduced as a consequence of repeated oxidations on the triterpene backbone in order to enhance its structural diversity.

Throughout this biosynthesis process of triterpenoid, steroidal saponins, and steroidal glycoalkaloids, the key classes of enzymes are oxidosqualenecyclase, P450-dependent monooxygenase, and UDP-dependent glycosyltransferases. Additionally, numerous transferases and other tailoring enzymes also play a significant role. The saponins' biosynthesis pathway is illustrated in Fig. 10.3.

10.3 In Vitro Production of Saponins

In vitro technique is unique approach for the production of plant secondary metabolites. There are various reports regarding the production of secondary metabolites from plants through in vitro culture of plant tissues/explants (Verpoorte et al. 2002; Murthy et al. 2014a). Saponins are one of the major pharmaceutically important compounds found in various parts of the plant spp. and various in vitro cultures such as shoot, callus, cell, and root cultures reported the presence of saponins (Murthy et al. 2014a; Biswas and Dwivedi 2019). This section is concentrating on the production of saponins via shoot culture, callus culture, cell suspension culture, adventitious root culture, and hairy root culture (Table 10.2).

10.3.1 Shoot Culture

In vitro shoot culture is one of the best-known tissue culture methods to isolate saponins. Some important plant spp. producing saponins via shoot cultures have been presented. Praveen et al. (2009) established *Bacopa monnieri* shoot culture in both semisolid and liquid medium and evaluated the bacoside A content and found

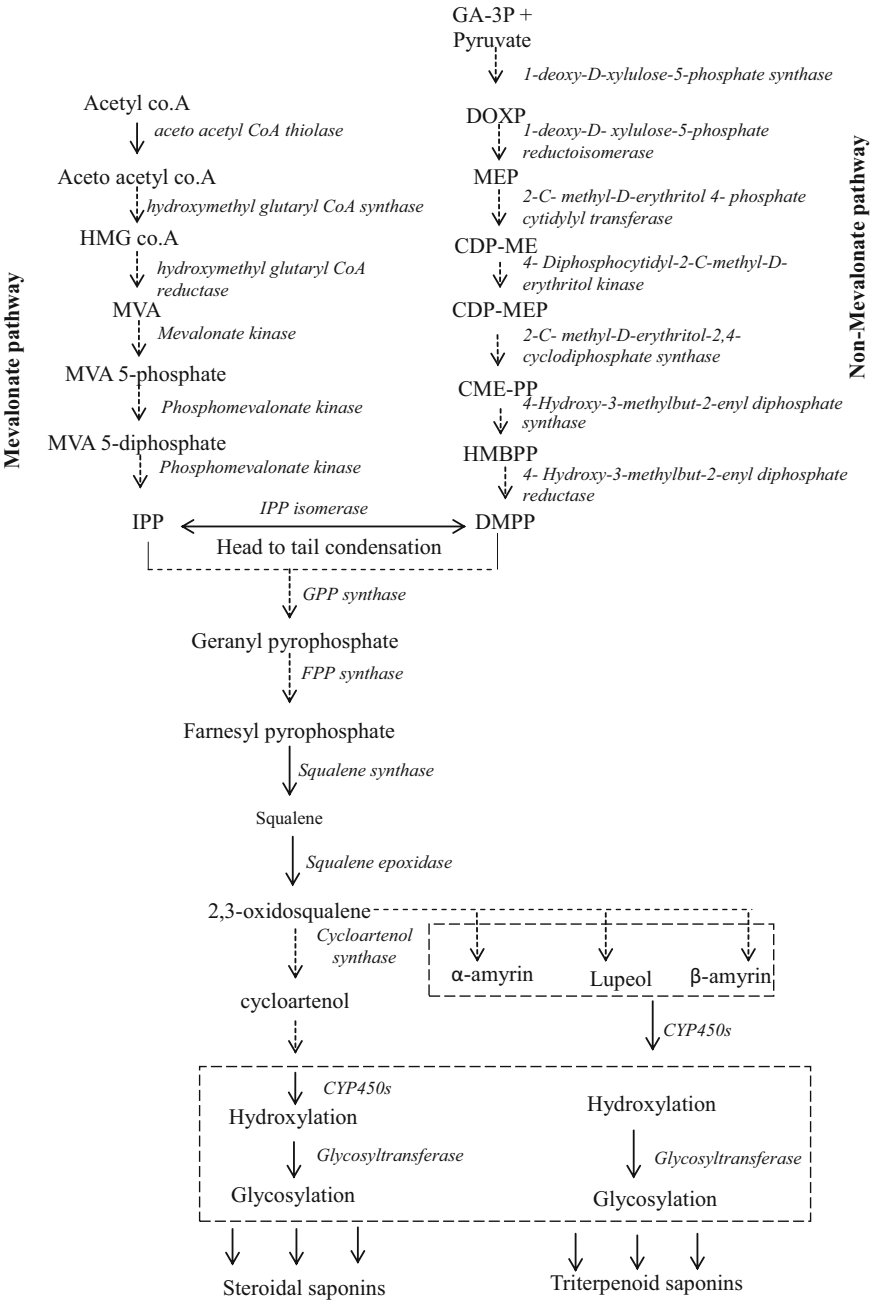

Fig. 10.3 Biosynthetic pathway of Saponins. (Source: Reproduced from Kumar et al. 2016)

Table 10.2 List of various explants and culture system used for the production of saponins

Sl. No.	Plant source	Explants	Culture system	Saponins	Reference
1	*Agave salmiana* Otto ex Salm-Dyck	Axillary shoot	In vitro plants	Tigogenin glycoside	Puente-Garza et al. (2017)
2	*Astragalus glycyphyllos* L.	Shoot	Callus, shoot, and suspension cultures	Cycloartane saponin	Shkondrov et al. (2019)
3	*Bacopa monnieri* (L.) Pennell	Leaf and stem	Shoot culture	Bacoside A	Sharma et al. (2013)
					Sharma et al. (2015)
		Aerial part	Shoot culture	Triterpenoid saponin glycosides	Watcharatanon et al. (2019)
		Leaf	Cell suspension culture	Bacosides	Koul and Mallubhotla (2020)
4	*Calendula officinalis* L.	Seedlings and Young leaf	Cell suspension culture	Oleanolic acid	Wiktorowska et al. (2010)
		Mature embryo	Hairy root culture		Alsoufi et al. (2019a)
					Alsoufi et al. (2019b)
5	*Centella asiatica* (L.) Urban	Node	Shoot culture	Asiaticoside	Prasad et al. (2013)
		Leaf	Callus culture	Centellosides	Mangas et al. (2008)
		Leaf	Cell suspension culture		Bonfill et al. (2011)
		Leaf	Hairy root culture		Kim et al. (2010)
6	*Chlorophytum borivilianum* Santapau and R. R.Fern	Leaf sheath	Callus culture	Stigmasterol and Hecogenin	Bathoju and Giri (2012)
7	*Codonopsis pilosula* Franch.	Seedling	Hairy root culture	Total saponins	Yang et al. (2020)
8	*Eryngium campestre* L.	Epicotyl	Shoot culture	Triterpenoid saponins	Kikowska et al. (2016)
9	*Eryngium maritimum* L.	Apical and axillary buds	Adventitious root cultures	Triterpenoid saponins	Kikowska et al. (2014)
10	*Eryngium planum* L.	Axillary buds	Shoot/callus/ cell suspension	Triterpenoid saponins	Kikowska et al. (2019)

(continued)

Table 10.2 (continued)

Sl. No.	Plant source	Explants	Culture system	Saponins	Reference
11	*Gymnema sylvestre* R. Br.	Leaves and stalks	Cell suspension culture	Gymnemic acids	Chodisetti et al. 2015, Chodisetti et al. (2013)
		Cotyledons and young leaves	Hairy root culture		Nagella et al. (2013), Praveen et al. (2014)
12	*Helicteres angustifolia*	Young leaves	Callus suspension cultures	Total saponins	Yang et al. (2019)
13	*Panax ginseng* C.A. Meyer	Stem	Cell suspension culture	Ginsenoside	Huang et al. (2013)
					Huang and Zhong (2013)
		Root	Adventitious root cultures		Wang et al. (2013)
			Hairy root cultures		Liang et al. (2009)
14	*Panax quinquefolium* L.	Seedlings	Hairy root cultures	Ginsenoside	Kochan et al. (2018)
15	*Panax vietnamensis* Ha & Grushv.	Leaf segments	Callus culture/ in vitro plants	Ginsenoside	Nhut et al. (2015)
		Shoot	Hairy root culture	Majonoside R2, dammarane	Ha et al. (2016)
16	*Ruscus aculeatus* L.	Phylloclades, rhizomes, and seeds	Root-rhizome culture	Ruscogenin	Khojasteh et al. (2019)
17	*Silene vulgaris* (Moench) Garcke	Leaf	Hairy root culture	Segetalic acid and gypsogenic acid	Kim et al. (2015)
18	*Zingiber montanum* (J. König) Link ex A. Dietr.	Rhizome buds	Callus/cell suspension	Total saponins	Rajkumari and Sanatombi (2020)

maximum in shoots cultured in liquid medium (2.2-fold) when compared to shoots grown on semisolid medium. Effect of various concentrations of sucrose and pH on in vitro shoot culture and bacoside A production in *Bacopa monnieri* was examined by Naik et al. (2010) and reported lower concentrations of sucrose and that pH treatments enhance the production of bacoside A. Continuation of earlier work, Naik

et al. (2011) studied the effect of macroelements and nitrogen source, and an increased amount of bacoside A content was reported in the shoot cultures grown in the higher concentration of nitrogen. Heavy metals such as manganese, zinc, and copper also affect the production of bacoside A in the shoot culture of *Bacopa monnieri* (Naik et al. 2015). Various media, medium strength, and carbon source alter the accumulation of bacoside A content in the shoot culture of *Bacopa monnieri* and found maximum in the treatments with full-strength MS medium, 2% sucrose and carbon source in combination with glucose and fructose (Naik et al. 2017). Prasad et al. (2013) worked on the accumulation of biomass and asiaticoside in *Centella asiatica* multiple shoot culture using fungal elicitors at different doses and culture age. Treatment with 3% v/v culture filtrates of *Trichoderma harzianum* in a culture medium on the tenth day influenced the biomass and asiaticoside accumulation by 2.53 and 2.35-fold when compared to untreated shoots in the culture cycle of 35 days. Interestingly, Nhut et al. (2015) showed that light-emitting diodes influence the accumulation of ginsenosides in the in vitro plant culture of *Panax vietnamensis.* Shkondrov et al. (2019) found in vitro shoot culture of *Astragalus glycyphyllos* yields double the amount of cycloartane saponins when compared to wild grown plants.

10.3.2 Callus Culture

In tissue culture, callus is an undifferentiated mass of cells that serves as the basic structure and the most important stage for development of embryos, shoots/roots, and friable cells by modifying the cultural conditions. The callus is also a source of secondary metabolites of particular plant spp. from which it has originated. Researchers have induced the callus culture of *Centella asiatica* for the production of centellosides and also studied the genes responsible for the biosynthesis of centellosides and found expression of the 5.8S rRNA gene (Mangas et al. 2008). In a callus culture of *Eryngium planum,* the application of methyl jasmonate elicitor in Murashige and Skoog (MS) medium fortified with 3% sucrose accumulated 1.2-fold triterpenoid saponins when compared to untreated callus culture (Kikowska et al. 2019). Yang et al. (2019) investigated the *Helicteres angustifolia* callus suspension culture for the estimation of phytochemical contents and found the potential source of total saponins in the culture.

10.3.3 Cell Suspension Culture

Cell suspension culture creates the avenue to obtain plant-based metabolites. It has the advantage over other culture method as it gets even/sufficient quantities of nutrients and cultural conditions, which induces the cells to grow faster and to maintain stability. *Bacopa monnieri* cell suspension cultures were tested with various elicitors (salicylic acid and jasmonic acid) and precursors (sodium nitroprusside, calcium pantothenate, and cholesterol) at different concentrations for the induction

of bacoside content, and in elicitor treated cultures, elevated biomass and bacoside content was observed on 6^{th}–9^{th} day (Koul and Mallubhotla 2020) and salicylic acid found to be the best suitable for the induction of bacoside content among the tested elicitors and precursors. Shkondrov et al. (2019) determined the cycloartane saponins' accumulation in suspension culture of *Astragalus glycyphyllos*. Wiktorowska et al. (2010) studied the effect of various elicitors (Jasmonic acid, chitosan, yeast extract, pectin, and fungal strain *Trichoderma viride*) on the production of oleanolic acid using cell suspension cultures of *Calendula officinalis*. Cell suspension cultures treated with jasmonic acid after 72 h found to be the most efficient elicitors used and accumulated 9.4-fold oleanolic acid when compared to untreated culture. In another study, biotic elicitors (extracts of *Agrobacterium rhizogenes, Aspergillus niger, Bacillus subtilis, Escherichia coli,* and *Saccharomyces cerevisiae*) were used to induce gymnemic acids from the cell suspension culture of *Gymnema sylvestre* and all the elicitors treated cultures showed positive response on the accumulation of gymnemic acids (Chodisetti et al. 2013). In continuation of earlier study, researchers applied methyl jasmonate and salicylic acid in cell suspension culture and yielded optimum gymnemic acid content at 72 h after methyl jasmonate treatment (Chodisetti et al. 2015).

10.3.4 Adventitious Root Culture

Adventitious root culture as a differentiated organ culture serves as an excellent system for the production of secondary metabolites as it grows relatively fast and stably without any harmful molecules (Murthy et al. 2014b). Kikowska et al. (2014) performed an experiment with adventitious root cultures of *Eryngium maritimum* to test the nutritional factor and plant growth regulators on the production of saponins. Results found that elevated accumulation of triterpenoid saponins of about 3.2-fold in in vitro-derived roots was observed compared to field grown plant roots. Adventitious root cultures grow continuously in the liquid media and presence or absence of exogenous auxins does not affect. Wang et al. (2013) studied the effect of methyl jasmonate in adventitious root cultures of *Panax ginseng* for the production of ginsenoside content, and 10 mg/L methyl jasmonate for 24 h treatment increased the accumulation of ginsenoside with 4.76-fold higher than control.

10.3.5 Hairy Root Culture

Transformed hairy roots are induced by infecting the explants with *Agrobacterium rhizogenes*, which has the higher capacity biosynthesis of secondary metabolite compared to non-transformed roots (Chandra and Chandra 2011). Kim et al. (2015) induced hairy root culture using leaf explants of *Silene vulgaris*, and analysed the triterpenoid saponins like segetalic acid and gypsogenic acid accumulation was found to be 5 and 2-fold higher after the treatment of methyl jasmonate respectively when compared to the control roots. *Panax vietnamensis* is an important plant

considering its peculiar pharmacological active saponins, namely majonoside R2 and dammarane. Hairy root culture of *P. vietnamensis* contains ginsenosides and majonoside R2, and dammarane saponins (Ha et al. 2016). Ginsenosides were induced in hairy root cultures *Panax quinquefolium* using trans-anethole elicitor, and trans-anethole activates the synthesis of saponins irrespective of exposure time (Kochan et al. 2018). Hairy root culture of *Calendula officinalis* is subjected to elicitor treatment (jasmonic acid and chitosan) for the stimulation of triterpenoid biosynthesis (Alsoufi et al. 2019a). Elicitor such as jasmonic acid, was very effective with respect to the accumulation of 20-fold oleanolic acid saponins in the hairy root tissue and 113-fold in the medium. Alsoufi et al. (2019b) selected abiotic elicitors (cadmium and silver ions, UV-C irradiation, and ultrasound) to induce triterpenoid biosynthesis from hairy root culture of *Calendula officinalis.* Heavy metals, UV-C irradiation, and ultrasound stimulated 12-fold, 8.5-fold, and 11-fold of triterpenoid biosynthesis, respectively. Recently, Yang et al. (2020) established the hairy root culture of *Codonopsis pilosula* and determined the total saponins' content from the grown hairy roots.

10.4 Bioreactors: Scale-up Techniques

The secondary metabolites production requires the optimum cell growth in the cell/organ culture. Adequate nutrients, balanced mixing, and oxygen supply minimize the plant cell damage which also depends on the species and cell lines selected (Georgiev et al. 2013). Other parameters such as temperature, pH, oxygen concentration, carbon dioxide, and substrate concentrations also control the production of secondary metabolites. Bioreactors in general possess the basic function which provides low shear stress, sufficient oxygen supply, and better mixing system of cells to maintain their optimum physiological conditions and regulate the metabolism of different environmental factors (Murthy et al. 2014b). Bioreactors are the important tools in the field of bioprocessing industry, which allow the optimum rate of multiplication of quality grade metabolites in a short duration of time with lesser cost. Various kinds of bioreactors are utilized for the production of secondary metabolites/saponins among stirred tank bioreactor (STB) which is most common. The advantage of these bioreactors is that they provide enough space for the cells to accumulate in different stages and also have the capacity to scale up nutrients because of their huge sizes (Gantait et al. 2020). For large-scale production of secondary metabolites/saponins, airlift bioreactors (ALB's) are also selected as one of most favourite bioreactors by researchers and industrialists as they provide maximum oxygen transfer and reduced cell shearing, which in turn give maximum yield (Gantait et al. 2020). For the reference purpose, some of the bioreactor types, culture system, and saponins are listed in the Table 10.3.

Applications of bioreactor enhance the multiplication of *Bacopa monnieri* in in vitro shoot culture rate and which in turn lead to propagate at commercial level (Saha et al. 2020). Jain et al. (2012) investigated shoot culture of *Bacopa monnieri* using nodal explants and found increased biomass accumulation when 10% aeration

Table 10.3 List of various types of bioreactors and culture system used for the production of saponins

Sl. No	Plant source	Culture system	Bioreactor type	Saponins	Reference
1	*Astralagus membranaceous* (Fisch.) Bunge	Hairy root culture	Airlift bioreactors	Astragalosides	Ionkova et al. (2010)
2	*Bacopa monnieri* (L.) Pennell	Shoot culture	Airlift bioreactors	Bacosides	Sharma et al. (2015)
					Saha et al. (2020)
3	*Centella asiatica* (L.) Urban	Cell suspension cultures	Bioreactor	Centellosides	Loc and Nhat (2013)
4	*Glycyrrhiza glabra* L.	Hairy root culture	Stirred tank bioreactor	Glyrhizzin	Mehrotra et al. (2008)
5	*Panax ginseng* C.A.Mey	Adventitious root cultures	Bioreactor (with sparging air)	Ginsenosides	Jeong et al. (2009)
		Cell suspension and adventitious root cultures	Stirred tank bioreactor, airlift bioreactors, bubble bioreactor		Murthy et al. (2014a, b)
					Murthy et al. (2017), Adil and Jeong (2018)
		Hairy root cultures	Airlift bioreactors, bubble bioreactor		Gantait et al. (2020)
6	*Panax quinquefolium* L.	Cell suspension cultures	Stirred tank bioreactor	Ginsenoside	Wang et al. (2012)
7	*Solanum chrysotrichum* (Schldl.)	Cell suspension cultures	Airlift bioreactors	Antifungal saponins	Salazar-Magallón and de la Peña (2020)

was applied in Growtek® bioreactor. In another study, two different bioreactors, Growtek® bioreactor and ALB, were used for bacosides production from *Bacopa monnieri* in in vitro shoot cultures, and optimum bacoside content was obtained in the biomass cultured in ALB system when compared to Growtek® bioreactor culture. As ALB provides enough aeration, it supplies the maximum oxygen for the synthesis of bacosides (Sharma et al. 2015).

Embryogenic tissues of *Panax ginseng* were cultured in two different types of bioreactors, STB and ALB, using flat-blade turbine and a paddle impeller; interestingly, higher biomass and optimum ginsenoside saponins production was observed in the ALB compared to STB (Asaka et al. 1993). The use of STB in a two-stage culture mode of cell suspension culture of *Panax quinquefolium* yields maximum

ginsenosides (Wang et al. 2012). Using 5-L bioreactor, Loc and Nhat (2013) standardized the protocol for the production of asiaticoside in cell suspension culture of *Centella asiatica*, the parameters included rate of aeration, size of inoculum, and speed of agitation. Recently, Salazar-Magallón and de la Peña (2020) carried an experiment for the production of antifungal saponins with a transformed cell line from cell suspension culture of *Solanum chrysotrichum* using ALB and found in vitro and in vivo antifungal activity of saponins against fungal pathogens.

Kim et al. (2004) studied the adventitious root cultures of *Panax ginseng* using various vessels (cone, bulb, balloon, and cylinder type) in ALB's and proved the balloon type of ALB is most appropriate for the accumulation of biomass and ginsenosides. Kim et al. (2005) worked on the aeration rate and sparger pore size and diameter and found great influence on the ginsenoside accumulation. Paek et al. (2009) found that ALB's are most suitable bioreactors for the production of biomass and ginsenosides from the adventitious root cultures of *Panax ginseng*. Different bioreactors, STB's and bubble column bioreactors (BCB's) with various capacities, were applied for the production of ginseng hairy root culture by Jeong et al. (2003) and maximum yield of hairy roots in 5-L and 19-L BCB's with 38 and 55-folds increment of biomass was obtained on 40 and 39 days of culture, respectively. Palazón et al. (2003) carried an experiment for the production of ginsenosides from ginseng hairy roots; it includes wave bioreactors or spray bioreactors, culture period, and medium exchange. In this study, wave bioreactor emerged as a promising system to grow hairy roots and found 28-fold biomass accumulation and enhanced ginsenoside content at the end of 56 days of culture when medium exchange was carried for every 14 days. Yu et al. (2003) found ALB's are the most promising bioreactors for the production of ginsenosides from ginseng hairy root culture.

10.5 Extraction and Detection Techniques of Saponins

Saponins are being isolated from different plant sources for their diversified economic and pharmacological activities. The saponins are distributed all over the plant body right from aerial parts and root regions. Presence of different functional groups and sugar units bound to the aglycone component of the saponins makes it very difficult in extraction technique. There are chances in which saponins may get hydrolysed and esterified in extractions. So it is bit challenging in the extraction of saponins (Runner 2006). Quantification of saponins is done similar to that of other metabolites using spectroscopic and chromatographic methods. For isolating the pure saponins from the plant source, preparative liquid chromatography is employed (Kim and Park 2001). But, for the estimation, HPLC (High-performance liquid chromatography) is the most accepted technique.

10.5.1 Extraction Techniques of Saponins

Right from initial times, researchers were using conventional methods to isolate the saponins from different plant sources. Different plant sources show different efficiencies with respect to the various extraction methods.

10.5.1.1 Conventional Methods

Soxhlet extraction was carried out using various types of solvents suitable for particular plant sources. There are many reported research articles which used soxhlet extraction for the isolation of saponins (Bajad et al. 2019). Different parameters influence the extraction procedure like time taken to extract the metabolites from the plant sample, nature of solvent, and its boiling point. In this extraction, the sample will be placed in the porous chamber made out of cheese cloth and vapours of the desired solvents are sent through it which ultimately takes out the target metabolites from the tissue. Polar solvents like water, ethanol, and methanol are suitable for the extraction of saponins through soxhlet extraction (Cheok et al. 2014). Reflux extraction is another method of extraction of metabolites where the plant sample is always kept in contact with desired polar solvents and subjected to continuous boiling, and once desired change and time are reached, it can be filtered and the extracts are used for the quantification of the desired metabolites with specific techniques (Tao et al. 2013). Maceration is also employed for extracting saponins and the principle behind it is solid-liquid extraction (Takeuchi et al. 2009). In this technique, the sample is soaked along with the desired solvents and chemicals for the required time, temperature, and stirring to aid the extraction of the metabolites (Verza et al. 2012). Solvent partitioning (liquid-liquid isolation) is also employed to effectively isolate the saponins (Kim and Park 2001; Cheok et al. 2014). Along with the above-mentioned conventional methods, there are many advanced techniques which can help in extraction of desired saponins. These techniques are environment-friendly and help us to conserve solvents. The main advantage is that the time taken for the isolation of the metabolites is comparatively less when compared to conventional methods.

10.5.1.2 Advanced Techniques

In ultrasound-assisted extraction, specific frequencies of sound waves are given to the tissue which helps us to extract the desired metabolites. Conceptually, it is similar to that of sonication. The time given for the extraction, solvent, and the frequency are the important parameters. The time taken for the extraction of saponins and related compounds is relatively less when compared to conventional methods like soxhlet extraction (Jadhav et al. 2009). Microwave-assisted extraction is most accepted technique for the isolation of metabolites in the recent times (Deore et al. 2015). The time required for the extraction of desired metabolite is comparatively less when compared to other techniques, usually the time taken for extraction will be around few minutes (6–8 min). Microwaves are given for the sample which is already associated with desired polar solvents like ethanol and methanol where extraction becomes very easy (Kerem et al. 2005). Accelerated solvent extraction

is another technique employed to isolate saponins and other metabolites (Zhang et al. 2013). The conventional and advanced techniques employed for extraction studies have been mentioned in Table 10.4.

10.5.2 Techniques for Detection of Saponins

Detection of saponins from the plant sample is done both qualitatively and quantitatively. Qualitatively to detect the saponins from the plant sample, foam test is conducted. If the foam persists for more than 15 min it confirms, the presence of saponins (Tadhani and Subhash 2006). Quantitatively, it is detected using chromatographic and spectrophotometric techniques.

Chromatographic techniques are employed for the quantification of plant secondary metabolites. In the same way, saponins are also quantified using high-performance liquid chromatography (HPLC) and are most widely accepted. Type of column used, flow rate, wavelength, and solvents used in the mobile phase are the important parameters considered for the quantification of saponins. Chromatogram and the peaks graduated help us to understand the presence of desired metabolites. Furthermore, these chromatographic techniques are modified by adding different components like mass spectroscopy, diode array detection, and evaporative light scattering detector, which help us to study the isolated molecules at atomic level (Guajardo-flores et al. 2012). Acetonitrile and water are most widely used solvents for eluting saponins from the samples.

In the spectrometric method, measuring the colour developed upon chemical reaction between desired metabolite and chemicals is the basic principle. Chemical standard used and wavelength at which colour measured are the important parameters in spectrometric methods. Vanillin (8%) along with sulphuric acid (72%) develops red-purple colour with plant sample which is later measured, which proves the presence of saponins (Le et al. 2018). The details of some of the detection methods for saponins are mentioned in Table 10.5.

Since saponins have such diversified economic and pharmacological uses, scientific community should design advanced techniques for the extraction of metabolites at large scale with minimum expenses. In the same way, the quantifying techniques can also be revised which can give more information of the desired molecule at less expenses.

10.6 Biological Activities of Saponins

Among various phytochemicals of the plant kingdom, saponins constitute an important class of bioactive molecules; this is attributed to the numerous biological activities exhibited by different aglycones of saponins, majorly the steroidal and the triterpenoid saponins (Bruneton 1999). These activities range from antitumor, gastroprotective, and antihyperlipidemic potential to their ability to regulate blood glucose and promote bone marrow haematopoiesis.

Table 10.4 Extraction techniques for saponins

Sl. No	Plant source	Part used	Saponin type	Extraction method	Reagents	Reference
1	*Bupleurum falcatum* L.	Dried roots	Saikosaponin c, a, and d	Solvent partitioning	70% ethanol- for extraction of the sample, diethyl ether, n-butanol, acetone and methanol- for solvent partitioning of the sample and LC	Kim and Park (2001)
2	*Panax ginseng* L.	Roots	Ginsenosides	Ultrasound-assisted extraction for 2 h using ultrasound bath (38.5 kHz) or ultrasonic probe (20 kHz)	Methanol, aqueous n-butanol, and 10% methanol- for extraction, acetonitrile- for TLC and HPLC	Wu et al. (2001)
3	*Cicer arietinum* L.	Seed powder	Soyasaponin B	Microwave-assisted extraction (2450 Mhz) for 20 min	Methanol, ethanol, butanol or aqueous ethanol, and butanol- for extraction of the sample, water and methanol- for HPLC	Kerem et al. (2005)
4	*Vigna radiata* L.	Whole plant	Soyasaponins	Soxhlet extraction for 24 h	Chloroform, 80% ethanol, butanol- for extraction of the sample	Waller et al. (1999)
5	*Ziziphus mauritiana* L.	Leaves	Saponin	Reflux extraction for 48 h	70% methanol and distilled water- for extraction of the sample	Dubey et al. (2019)
6	*Glycyrrhiza glabra* L.	Root powder	Triterpene saponins	Reflux extraction	50% aqueous methanol- for extraction of the sample	Tao et al. (2013)
	Glycyrrhiza inflate Bat.					
	Glycyrrhiza uralensis Fisch.					
7	*Momordica charantia* L.	Vine and leaves	Triterpene saponins	Reflux extraction	Ethanol- for extraction of the sample	Chen et al. (2009)
8	*Momordica charantia* L.	Root powder	Triterpene saponins	Maceration for 6 h at 60 °C	Methanol- for the first digestion, ethanol, n-butanol- for the subsequent maceration	Chen et al. (2008)

(continued)

Table 10.4 (continued)

Sl. No	Plant source	Part used	Saponin type	Extraction method	Reagents	Reference
9	*Ipomoea batatas* L.	Tuber flour	Triterpene saponins	Maceration for 4 h at room temperature	80%methanol- for the maceration	Dini et al. (2009)
10	*Tribulus terrestris* L.	Whole plant powder	Steroid saponins	Maceration for 1 h followed by reflux extraction for 3 times around 1 h	Chloroform- for maceration, 70% ethanol- for reflux extraction	Dinchev et al. (2008)
11	*Bacopa monnieri* (L.) Pennell	Plant dry powder	Triterpenoid saponins	Ultrasound sonication for 10 min	Methanol- for the extraction of the sample	Ganzera et al. (2004)
12	*Ziziphus jujuba* Mill.	Leaves dry powder	Zizyphussaponins I, II	Ultrasound sonication at 40 kHz for 30 min at room temperature	80% ethanol- for the extraction of sample	Guo et al. (2011)
	Ziziphus jujuba var. spinosa Bunge.					
13	*Panax notoginseng* (Burkill) F.H. Chen	Dried powder	Notoginseng saponins	Microwave-assisted extraction (2450 MHz) for 6 min	Water saturated n-butanol- for the extraction of the sample	Vongsangnak et al. (2004)
14	*Litchi chinensis* Sonn.	Seed powder	Litchinoside and Sapondoside	Liquid-phase pulsed discharge and ultrasonic extraction (276 W at 47 °C), 3 mm hollow electrode, 123 ml/min flow velocity.	30% ethanol- for the extraction of the sample	Fan et al. (2020)
15	*Gymnema sylvestre* R. Br.	Dry leaf powder	Oleanolic acid	Microwave-assisted extraction (2450 MHz) for 8 min	Ethanol- for the extraction of the sample	Mandal and Mandal (2010)
16	*Glycyrrhiza glabra* L.	Dry powder	Triterpenoid saponins	Ionic liquid-based ultrasonic-assisted extraction along with in situ		Ji et al. (2020)

				alkaline aqueous biphasic system for 20 min.	NaOH and $[C_4MIM]BF_4$ (imidazolium type)-for alkaline aqueous biphasic system	
17	*Trigonella foenum-graecum* L.	Seed dry powder	Common saponins	Microwave-assisted extracted	40–80% ethanol- for the extraction	Akbari et al. (2020)

Table 10.5 Estimation methods for saponins

S. No	Plant source	Part used	Saponin type	Estimation method	Reagents	Yield	Reference
1	*Bupleurum falcatum* L.	Dried roots	Saikosaponin c, a, and d	HPLC C18 column flow rate- 1 mL/min wavelength- 203 nm	70% ethanol- for extraction of the sample, acetonitrile: Water- for eluting the samples	144.25 mg/100 g and 247.25 mg/100 g of saikosaponin c and d (145 days)	Park et al. (2000)
2	*Ilex paraguariensis* A.St.-Hil.	Aerial parts	Matesaponin 1, 2, 3, 4, 5	HPLC C18 column flow rate- 1 mL/min wavelength- 203 nm	Water- for extraction of the sample, acetonitrile: Water(70:30)- for eluting the samples	352 µg/ mL in saponin fraction	Gnoatto et al. (2005)
3	*Bryonia laciniosa* L.	Seed powder	Saponin	Spectrophotometric method at 538 nm	Ethanol- for extraction of the sample, vanillin (8%) + sulfuric acid (72%)- for colour development	15 µg/mg of oleanolic acid equivalent	Patel et al. (2012)
4	*Phaseolus vulgaris* L.	Dry powder	Soya saponins	HPLC-DAD-ELSD and TOF (diode array detection- evaporative light scattering detector and time of flight) flow- 0.5 ml/ minwavelength- 295 nm	80% methanol- for extraction of the sample, Trifluoroacetic acid and HPLC-grade acetonitrile- for eluting the samples	1063.62 mg/100 g in (soaked) and 455.95 mg/100 g in (raw)	Guajardo-Flores et al. (2012)
5	*Bacopa monnieri* (L.) Pennell	Dry powder	Bacoside and Bacopasides	HPLC column- C-8(2) flow rate-0.5 ml/min, wavelength- 205 nm.	Methanol- for the sample extraction water and methanol- for the eluting the sample	1.1 to 13% of dry weight	Ganzera et al. (2004)
6	*Asparagus species*						

		Freeze dried lyophilized powders of spears	Protodioscin, Saponins	HPLC-DAD−MS, particle size-5 µm flow- 0.3 mL/min	80% ethanol- for the sample extraction 1% formic acid and acetonitrile- for eluting the samples	1.09 to 2.73 mg/ 100 g of fresh weight	Vázquez-Castilla et al. (2013)
7	*Tribulus terrestris* L., *Achyranthus aspara* L., *Albizia lebbeck* (L.) Benth.	Seed dry powder	Quillaja saponin	Spectrophotometric method at 544 nm	Petroleum ether and methanol- for the extraction of the sample, vanillin (8%) + sulfuric acid (72%)- for colour development	25.65, 45.75, and 48.26% (w/w)	Goel et al. (2012)
8	*Solanum nigrum* L.	Dry leaf powder	General sapogenins	Ultra-high-performance liquid chromatography-MS flow rate- 0.4 mL/min	Water and acetonitrile, 0.1% formic acid- for eluting the sample	0.4 to 0.8% of dry mass	Yuan et al. (2019)

Saponins are amphiphilic molecules, consisting of both hydrophilic and hydrophobic moiety. The structural complexity, particularly the molecular structure, enables it to interact with the lipid components of the plasma membrane including sterols, proteins, and phospholipids and these interactions result in the formation of aggregates; this property majorly accounts for the wide range of biological activities that saponins have (Lorent et al. 2014) (Table 10.6). In addition to this, the ability of saponins to undergo chemical changes during processing or storage and their glycosylation pattern also determine the biological activities (Augustin et al. 2011; Güçlü-Ustündağ and Mazza 2007). Antifungal, anti-parasitic, antibacterial, and antioxidant are few of the minor activities of saponins, in addition to the activities mentioned below:

10.6.1 Anti-tumor Activity

One of the most important biological activities of saponins include antitumor activity. This property is exhibited by major saponins like dammarane or oleanane as well as their derivatives such as theasaponin. The activity is operated by different mechanisms by different classes of saponins, some of the major ones include reducing the synthesis of DNA, causing damage to DNA, inhibiting tumour angiogenesis, altering the host susceptibility to mutations, and by increasing immunosurveillance and apoptosis (Shibata 2001). Saponins are found to act on some of the major types of cancers such as breast cancer, lung cancer, liver cancer, colon cancer, and gastric cancer. The impact of aglycones on the antitumor activities has been studied and it is concluded that factors such as number of hydroxyl groups (Wang et al. 2007), site of hydroxyl group, lipophilicity of sugars (Mimaki et al. 2001), and sequence of sugars present (Bang et al. 2005) affect the activity of saponins. According to a study, spirostanes such as polyphyllin D show strong anticancer activity by inducing endoplasmic reticulum stress-mediated apoptotic pathway by the accumulation of unfolded or misfolded proteins, followed by mitochondria-mediated pathways by the downregulation of anti-apoptotic and upregulation of pro-apoptotic factors, which eventually leads to the apoptosis of tumour cells (Siu et al. 2008; Cheung et al. 2005); dioscin shows similar activity in addition to the antiproliferative activity against cancer cells (Wang et al. 2006). In contrast to this, dammarane saponins like OSW 1 damage the mitochondria and its cristae and triggers the calcium-dependent apoptotic pathway (Zhou et al. 2005). The cytotoxic effect of avicin D has been demonstrated by Haridas et al. (2009) where it was shown that saponin downregulates some of the major factors involved in apoptosis such as cyclin D1, c-myc, VEGF, and Bcl-2; this is done by decreasing the level of IL-6 and dephosphorylation of Stat-3. Platycodon D is another antineoplastic agent that operates by generating reactive oxygen species and activating CASPASE 3, thereby inducing apoptosis (Shin et al. 2009). Few other mechanisms underlying antitumour activity include inhibition of COX-2/PGE-2 pathway (Han et al. 2013), upregulation of proapoptotic proteins like Bcl-2 and Bax, generation of ROS, inhibition of wnt/beta catenin signalling pathway, downregulation of few other

Table 10.6 Saponins and their pharmacological activities

Sl. No.	Saponins	Activity	Mechanism	Reference
1	Notoginsenoside R1	Anti-atherosclerosis	Inhibition of plasminogen activator inhibitor 1	Zhang & Wang (2006)
2	Ginsenosides	Anticoagulant activity	Increasing the synthesis of plasminogen activators and antagonistic activity of platelet activating factors	Jung et al. (1998) Zhang et al. (1997)
3	Sea cucumber saponins	Anti-hyperuricemic activity	Inhibition of enzymes xanthine oxidase and adenosine deaminase	Xu et al. (2011)
4	Platycosides	Anti-inflammatory activity	Inhibition of NF-κB activation and MAPK signalling pathways	Jang et al. (2013)
5	Maesa Saponins, oleanolic saponin	Haemolytic activity	Bursting of erythrocyte membrane due to the interaction between sterols of membrane and saponins	Voutquenne et al. (2003) Baumann et al. (2000) Sindambiwe et al. (1998)
6	Cucumarioside A2-2, and Frondoside A	Immunomodulatory activity	Stimulation of cytosolic calcium concentration, lysosomal activity, ROS formation, and natural cellular defence barrier	Aminin et al. (2009)
7	Oleanolic acid	Anti-HIV activity	Inhibition of in vitro HIV-1 protease activity	Mengoni et al. (2002)
8	Ziyu glycoside	Promotion of bone marrow hematopoiesis	Reduction of suppressive cytokines and activation of FAK and Erk1/2 pathways	Chen et al. (2017)
9	Ginsenoside Rg 1	Neuroprotective activity	Inhibition of mitochondrial apoptotic pathway and increase in activity of choline acetyltransferase	Leung et al. (2007) Yamaguchi et al. (1997)
10	Oleanolic acid	Molluscicidal activity	Formation of pores resulting in leakage of liquids due to the interaction between saponins and cholesterol of the membrane	de Paula Barbosa (2014)

proteins like cyclin D, cdk-4, and MDM2, and upregulation of caspase 3/9, p21, p53 expression which eventually leads to cell cycle arrest or apoptosis (Wang et al. 2018; Cui et al. 2018). Furthermore, targeting the three main family members of MAPKs, namely, p38, JNK, and ERK, and inducing the formation of massive vacuoles containing lysosomes and autolysosomes that is characteristic of autophagy is another way of killing the cancer cells which is mainly performed by the saponin jujuboside B (Xu et al. 2014).

10.6.2 Anti-hyperglycemic Activity

Triterpene saponins such as saponins of *Panax ginseng*, Charantin, and saponins from *Asparagus officinalis* L., have shown the ability to improve the uptake of glucose and insulin sensitivity in the liver cells (Hu et al. 2014; Kim et al. 2009; Zhu et al. 2020). Ginsenoside Rb1 acts as a hypoglycemic agent by regulating glycolipid metabolism and increasing the insulin sensitivity, which are achieved by activation of the Peroxisome proliferator-activated receptor gamma (PPAR-γ), thereby improving glucose homeostasis (Kwon et al. 2012). In vitro studies have revealed that ginsenoside activates insulin signalling pathways such as phosphorylation of insulin receptor substrate-1 and protein kinase B (PKB) and the activity of phosphatidylinositol 3-kinase (PI3K); this in turn leads to the increased translocation of GLUT receptors in the adipose tissue (Shang et al. 2008). In addition to this, saponins target glucose metabolizing enzymes in order to normalize blood glucose level. Diosgenin is a steroid saponin which is known to promote glycogenolysis by increasing the levels of enzymes such as Phosphofructokinase and pyruvate kinase (Raju and Chinthapally Rao 2012. Lee et al. (2011) have reported that ginsenoside Rb2 inhibits gluconeogenesis by upregulating SHP (Short heterodimer partner), which inhibits the mRNA expression of gluconeogenic enzymes such as Glucose-6-phosphatases and phosphoenolpyruvate carboxykinase. The inhibitory effect of saponins against carbohydrate hydrolysing enzymes including pancreatic alpha amylase and alpha glucosidase and their potential to reverse the atrophic pancreatic beta cells as shown by Oleifera Saponin A1 (Di et al. 2017) further establishes saponins as an effective antidiabetic agent. Another hypoglycemic mechanism of saponins involves regulation of AMPK/NF-κB signal pathway, improvement of lipid metabolism in diabetic subjects, and inhibition of reactive oxygen species formation resulting in reduced oxidative stress and normal functioning of the kidneys (El Barky et al. 2016; Wang et al. 2019).

10.6.3 Anti-hyperlipidemic Activity

One of the common lipoprotein abnormalities involves decreased levels of HDL cholesterol and increased levels of LDL cholesterol and triglycerides (Gupta et al. 1994); these conditions are closely associated with diseases like coronary heart disease, atherosclerosis, and diabetes. Thus, modulating lipid metabolism or levels

of TC, TG, HDL, and LDL is one way of functioning as an antihyperlipidemic agent and this is efficiently carried out by saponins such as soyasaponins, ginsenosides, and trigonelline. One way of doing this is by influencing the lipid biosynthetic pathway; this mechanism was demonstrated by Hu et al. (2010), where the dietary saponins could inhibit the mRNA expression of SREBP-1c; inhibition of this transcription factor leads to a reduced expression of lipogenic genes like fatty acid synthase (FAS) and glycerol-3-phosphate acyltransferase (GPAT) (Horton et al. 2002), thereby inhibiting enzymatic activity and eventually decreasing lipid synthesis. Another mechanism is by increasing the activity of carnitine palmitoyl transferase (CPT), an important enzyme in beta oxidation of fatty acids; increased activity of CPT results in reduced triglyceride synthesis due to reduced flux of fatty acids. Hypocholesterolemia is another activity of saponins which is operated either by inhibiting the enzymes of cholesterol biosynthetic pathway such as 3-hydroxy-3-methyl-glutaryl-CoA reductase (Elekofehinti et al. 2012) or by increasing the dual transporters in the liver, namely ABCG55 and ABCG8, which transport free cholesterol into bile thereby reducing their level in the liver (Ji and Gong 2007). Further, activation of AMPK/ACC signalling pathway by total saponins (Xu et al. 2018) increased expression of lipoprotein lipase (Eu et al. 2010) and reduced expression of fatty acid binding protein 4 (FABP4) (Bhavsar et al. 2009), which are few other mechanisms involved in hypolipidemic effects of saponins.

10.7 Commercial Utilization and Prospects

Biotechnology makes possible to use the plant-based compounds and their derivatives in the pharmaceutical/nutraceutical industries. It is necessary to follow the suitable method of cultivation to achieve enhanced production of secondary metabolites from plants. Commercial scale bioreactors were manufactured by South Korean company Kihyung Plant Co., Ltd. The CBN Biotech Company, South Korea, used a commercial scale bioreactor to produce ginseng adventitious root. The company produces nearly 35 tons of adventitious roots of ginseng per year, which are used in industries like food, cosmetics, and pharmaceutical (Murthy et al. 2014b). This is one of the fine examples of application of biotechnological tool to meet the commercial need. By looking at the biosafety and toxicological evaluation of ginseng adventitious roots for the human consumption, the United States Food and Drug Administration (USFDA) and Korean Food and Drug Administration (KFDA), ISO (9001/2000), have approved (2,030,950, dated: 06/07/2002) products of ginseng adventitious roots and their commercial production. The ginsenosides are one of the most important saponins produced from ginseng roots which have the important medicinal value and high global market. These metabolites achieve the total revenue of 2 billion American dollars. United States of America, Canada, China, South Korea, Japan, and European countries are the major producers and commercial users of ginsenosides (Kim et al. 2013; Gantait et al. 2020). In Japan, company named NITTO DENKO CO. produces ginseng cell culture using large scale bioreactors (20,000 and 25,000 L) and markets the food products from ginseng

which are very healthy and nutritious since 1988 (Adil and Jeong 2018). Hairy roots are one of important sources of the saponins and other metabolites; the company named ROOTec Bioactives AG in Witterswil, Switzerland, is one such company which produces hairy roots at industrial level (Talano et al. 2012). The company produces high quality compounds using applied biotechnological tool to meet the consumers' need and world market, especially pharma and cosmetics. The compounds are more efficient and production is rapid and is of reasonable cost compared to chemical synthesis and conventional production. For the production of optimum/increased level of hairy roots biomass, ROOTec came with a new bioreactor named "ROOTec Mist Bioreactor" (Talano et al. 2012). Some of the saponin compounds which are used in the pharmaceutical field are Madessol®, Centellase®, and Blastoestimulina®. These extracts are used in the form of tablets, drops, ointments, powder, and injections (Gallego et al. 2014).

10.8 Conclusion and Recommendation

Saponins are plant-derived triterpene glycosides that have commercial applications in the food, pharmaceutical, and cosmetic industry. Saponins being a chief metabolite present in many medicinally important plants, there is a need to prove their pharmacological ability which in turn helps in formulating the medicines in high market demand. There is a huge demand for these metabolites (saponins) because of their pharmacological importance and there is a need for their extensive mass production using biotechnological applications. There are ample of research reports on the production of saponins via in vitro method, and their applications at the industrial/commercial level. With the help of plant tissue culture approaches like callus and organ cultures, the secondary metabolite production can be enhanced. In the recent times, bioreactors serve as important biotechnological tools for the metabolite enhancement at the cellular level. Further, saponins production can be elevated by the identification and manipulation of genes which encode the key enzymes and alter the gene expressions, which in turn can be achieved through metabolomics, proteomics, and transcriptomics. Metabolic engineering strategy can be applied for the enhanced production of metabolites in less time and space.

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