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THE BIOSYNTHESIS OF GINSENOSIDES: A MINI-REVIEW

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ABSTRACT

Ginsenosides, the bioactive constituents from the various *Panax* species are saponins. Saponins are a group of chemically diverse non-volatile natural compounds (secondary metabolites) that are mainly produced by plants and certain marine organisms such as starfish and Sea cucumbers. This mini-review gives a brief account of the general biosynthetic pathways shared by all saponins and then focuses on ginsenosides in particular. A knowledge of the biosynthetic pathway of ginsenosides could be very helpful in the cultivation of genuine ginseng breeds with the sole aim of obtaining the most bioactive compounds.

KEYWORDS: Ginsenosides, Panax, Saponins, biosynthetic pathways.

1. INTRODUCTION

The bioactive constituents from the various *Panax* species have been found to be saponins, referred to as ginsenosides. Saponins are a group of chemically diverse non-volatile natural compounds (secondary metabolites) that are mainly produced by plants and certain marine organisms such as starfish and Sea cucumbers.[1,2] The term 'saponin' is derived from the Latin word 'sapo' which means soap due to the fact that they form stable soap-like foams when shaken in water. This soap-like property is due to their amphiphatic nature. Saponins usually consist of a sugar moiety glycosidically linked to a hydrophobic aglycone known as sapogenin, which may be triterpenoid or steroid in nature. This linkage of the lipophilic aglycone to hydrophilic saccharide side chains further explains their 'soap-like' properties. The sugar moieties usually include glucose, galactose, glucuronic acid, xylose, rhamnose or methyl pentose. Their biological roles in plants though not fully understood, they have been considered to play part of the defense system. They are known to possess antimicrobial, fungicidal, allelopathic, insecticidal, and molluscicidal properties.[3] The production and accumulation of saponins is influenced by environmental factors such as light irradiation, water and nutrient availability or a combination of factors.[2,4] Due to the unique nature of saponins, they have the potential to be used industrially as preservatives, flavor modifiers and agents for removing of cholesterol from dairy products.[5] In general, saponins have been useful in the cosmetic, beverage and confectionery industries[6,7] and most importantly in the pharmaceutical industry. Saponins are widely placed under two groups, triterpenoid and steroid saponins. These types result from differences in the structure and biochemical background of their aglycone moieties (sapogenin).[8] Though both sapogenins are thought to be derivatives of the central metabolite in phytosterol anabolism, 2, 3-oxidosqualene, triterpenoid sapogenins branch off the phytosterol pathway by alternative cyclization of 2, 3-oxidosqualene. Steroidal sapogenins however are believed to be derivatives from the intermediates in the phytosterol pathway downward of the cycloartenol formation.[9,10]

Ginsenosides are traditionally classified into two categories based on the structural differences of their aglycone skeletons as the dammarane- and oleanane-types. Yang *et al.* reviewed all saponins from the *Panax* genus and provided a list of five groups into which they placed all the ginsenosides known as of 2012. They grouped ginsenosides into protopanaxadiol-type, protopanaxatriol-type, ocotillol-type, oleanolic acid-type, C17 side-chain varied type and miscellaneous.[11] Using the traditional classification, we have proposed a modified classification system which provides a wider scope and categorizes all ginsenosides with a reasonably good accuracy. Based on the structural differences of the sapogenins, ginsenosides can be grouped into; (1) dammarane-type ginsenosides and their derivatives (2) olenane-type ginsenosides and (3) miscellaneous. The dammarane-type ginsenosides can further be subdivided into the protopanaxadiol-type (PPD), the protopanaxatriol-type (PPT). The dammarane-type ginsenoside derivatives include, the ocotillol-type ginsenosides, the cleavage products of PPD- and PPT-types, hydration and dehydration products of PPD- and PPT-types, ginsenosides with epoxy group and peroxidation products of PPD- and PPT-types.[12] This subgroup could be widened to encompass methoxylation, carbonylation, cyclization, oxidation (at the double bond), degradation, rearrangement or even a mixture of reactions. This system of classification stays valid so long as the new compounds are derivatives of the PPD-/PPT-type skeletons.

Ginsenosides, being triterpenoid saponins share the general biosynthetic pathway of saponins.

2. Biosynthesis of saponins

Saponins are believed to be biosynthesized in plants as derivatives of metabolites from phytosterol anabolism. The biosynthetic pathway can be categorized into four distinct processes:

1. Biosynthesis of 2,3-oxidosqualene from acetyl-CoA
2. Cyclization of 2,3-oxidosqualene
3. Modification of cyclization product
4. Glycosylation of sapogenin

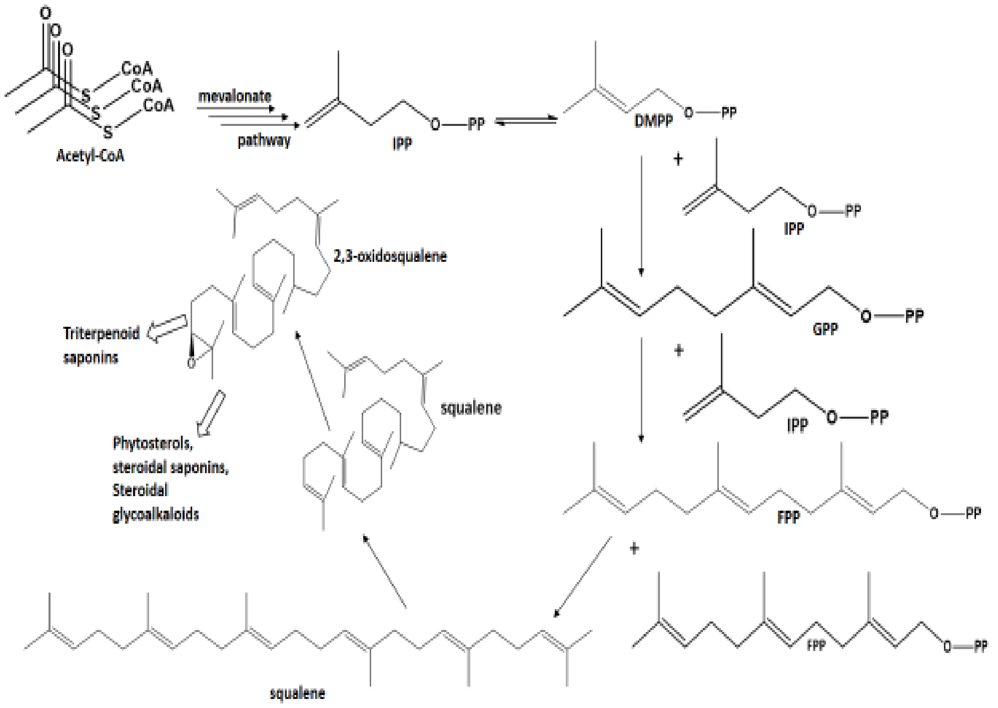

Fig: 1. Biosynthetic steps leading to the formation of the precursor 2,3-oxidosqualene. IPP–Isopentenyl pyrophosphate, DMPP –dimethylallyl pyrophosphate, GPP – geranyl pyrophosphate, FPP – farnesyl pyrophosphate.[2]

2.1. Biosynthesis of 2, 3- oxidosqualene from acetyl-CoA

The biosynthetic pathway begins with the condensation of two five-carbon building units known as 3-isopentylpyrophosphate (IPP) and dimethylallylpyrophosphate (DMAPP). IPP and DMAPP are either derived from the condensation of acetyl-CoA in the mevalonate pathway or the pyruvate and phosphoglyceraldehyde pathway (also known as the non-mevalonate pathway). The mevalonate pathway occurs in the cytosol while the non-mevalonate pathway occurs in the plasmids. The condensation product is a ten-carbon unit called geranyl pyrophosphate (GPP). GPP is further condensed with a second IPP unit to form fanesyl pyrophosphate (FPP), a fifteen-carbon unit. Two FPP units are linked head-to-tail forming a thirty-carbon unit known as squalene.[13] Squalene is then epoxygenated to 2, 3-oxidosqualene, a common precursor of triterpenoid saponins, steroidal saponins and phytosterols.[2,10,14]

2.2. Cyclization of 2, 3-oxidosqualene

This is considered the first committed step in triterpenoid saponins and phytosterol biosynthesis[2,15] catalyzed by enzymes called oxidosqualene cyclases. The type of cyclase involved in the cyclization reaction determines the skeleton that emerges. During this process, 2, 3-oxidosqualene is converted to cyclic derivatives through protonation and epoxide ring opening. This results in the formation of a carbocation that is prone to undergo several cyclization reactions. Subsequent rearrangements by a series of hydride shifts, methyl group movements result in the formation of new carbocations. The reactions of these new carbocations are terminated or neutralized by proton elimination to form a double bond, a cyclopropanyl ring or by reacting with water to give a hydroxyl group. Some oxidosqualene cyclases have been found to quench cyclization reactions by a stereospecific addition of water and forming saturated, dienolic cyclization products as the result.[16] The entire cyclization process results in the formation of mostly polycyclic molecules containing 5-membered and 6-membered rings due to the introduction of internal bonds into the oxidosqualene backbone.[2] Cyclization could happen in two ways either by ‘chair-chair-chair’ conformation or ‘chair-boat-chair’ conformation resulting in the formation of skeletons with different stereochemistry. ‘Chair-chair-chair’ conformations favor the biosynthesis of triterpenoid sapogenins while ‘chair-boat-chair’ conformations result in the formation of steroidal sapogenins.

Cyclization of the ‘chair-chair-chair’ conformation (enabled by protonation) leads to the formation of the 20-carbon tetracyclic dammarenyl carbocation. Saponins formed from this carbocation are termed *dammarane-type saponins*.[17-19] This carbocation forms the foundation within which other types of triterpenoid saponins could result. Through a series of chemical shifts and rearrangement, these saponins could result. For instance, an 8-carbon carbocation called tirucallenyl is formed from a cascade of hydride and methyl shifts in the dammarenyl carbocation. All saponins derived from the tirucallenyl carbocation are known as *tirucallane-type saponins*. Alternatively the dammarenyl carbocation could be rearranged to the baccharenyl carbocation. Subsequent annulation of the baccharenyl carbocation results in the formation of the pentacyclic lupanyl carbocation. Lupanyl carbocation forms the backbone of the *lupane-type saponins*. Ring opening and expansion of the pentacyclic E- ring of the lupanyl carbocation into a 6-carbon ring forms the germanicyl carbocation. This is an intermediate cyclization product which is further transformed into oleanyl carbocation, the origin of *oleanane-type saponi*ns. Germanicyl carbocation could also be transformed into the taraxasterenyl[20] or the ursanyl carbocations.[21,22] These form the backbone of *taraxasterane-type* and *ursane-type* saponins respectively. The ursane skeleton is also referred to as α-amyrin skeleton and is the cyclization product of α-amyrin synthase. The dammarenyl carbocation could also a shift of the C13-C17 bond forming a carbocation which is cyclized to C25 pentacyclic hopenyl carbocation. *Hopane-type* saponins are derived from this carbocation.[23,24]

Cyclization of the 2, 3-oxidosqualene in the ‘chair-boat-chair’ conformation also leads to a different type of sapogenin backbones. The cyclization process, also proton-initiated, initially produces a tetracyclic prostosteryl carbocation which undergoes a series of methyl and hydride shifts to form lanosteryl carbocation as the intermediate. This intermediate undergoes deprotonation of C19 methyl group resulting in the formation of cyclopropane ring as in cycloartenol. Saponins which are derivatives of this cycloartenol backbone are called *cycloartane-type saponins*.[25-27] Alternatively, the lanosteryl carbocation is further subject to a methyl and hydride shift forming cucurbitanyl carbocation and saponins resulting from this are classified *as cucurbitane-type* saponins.[28,29] The lanosteryl carbocation can also be deprotonated to form lanosterol. Saponins derived from lanosterol are grouped as *lanostane-type saponins*.[30] Lanosterol acts as an intermediate for the formation of another type of saponin. When lanosterol is demethylated and undergoes isomerization of the double bond, a cholesterol is formed. Saponins derived from this skeleton are known as *steroid-type saponins*.[31]

2.3. Modification of cyclization product

The cyclization products which form the basic backbone structures undergo modification (also known as decorations) before glycosylation. These modifications vary among different plant species and even among organs and tissues of same plants. Additions or otherwise of functional groups such as keto-, acetal, carboxyl and hydroxyl moieties at certain positions of the backbone structure represent the most common modifications. In some instances, these modifications form the basis for further decorations leading to diversified products. The DDMP saponin from pea as reported by Heng *et al*. was found to be a secondary modified product from the linkage of the basic skeleton with 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) group.[32] Another form of secondary modification is intramolecular bridge formations between adjacent functional groups i.e. ester, ether or acetal linkages. Changes in the backbone saturation after cyclization could be accounted for by the differences in the oxidosqualene cyclase-mediated termination process. It is believed that changes such as intramolecular bridge formations and addition of either simple or complex side groups after the primary modification involves enzymes of varied biochemistry. Most of these modifications are thought to be introduced by cytochrome P450 enzymes. Since these P450 enzymes are numerous (the activities of some not clearly understood), their mediated products are diverse in chemical structure.

It is also worth noting that several of the saponin skeletons have been found to undergo ring cleavage, homologation and degradation forming new skeletons as a result. For instance, the dammarane skeleton is found to produce 3, 4-seco[33] and 15, 16-seco[34] skeleton upon ring cleavage. The oleanane skeleton upon fragmentation can produce the 17, 22-seco[35] skeleton or degraded to form the 23-nor[36], the 27-nor[20], the 28-nor[37] and the 30-nor[29,38] skeletons.

2.4. Glycosylation of sapogenin

Glycosylation is considered the step that confers biological activities on saponins. Its significance in the biosynthetic pathway cannot be gainsaid. It involves the introduction of saccharide chain to the sapogenin backbone after the P450-catalyzed additions of polar moieties (hydroxyl or carboxyl groups). It is the final phase in the biosynthesis of saponins, though some exceptions exist.[39]

A typical triterpenoid glycosylation process consist of the linkage of oligomeric sugar chains, 2-5 monosaccharide units at positions C3 and/or C28 of the sapogenin backbone. The predominant sugar units are glucose, galactose, glucuronic acid, rhamnose, xylose and arabinose in the saccharide chains. Glycosylation is also presumed to involve a series of activities by a variety of enzymes of the family, 1 uridin diphosphate glycosyltransferases (known simply as UGTs).[2]

2.5 Summary of the Biosynthesis of Ginsenosides

Triterpenoid and steroidal saponins share the same precursor, 2, 3-oxidosqualene, a product of the isoprenoid pathway. Their biosynthesis are similar up to the point of 2, 3-oxidosqualene formation. Diversification occurs at the point of 2, 3-oxidosqualene cyclization.

Two biosynthetic pathways are known to be responsible for the formation of IPP and DMAPP, which are universal precursors for all isoprenoids; the mevalonate and non-mevalonate pathways. It is generally presumed that steroidal saponins are mostly formed through the mevalonate pathway while monoterpenes, diterpenes, carotenoids, phytol and plastoquinone are preferentially formed via the non-mevalonate pathway.[40,41] Zhao *et al*. reported that the biosynthesis of ginsenosides involves both pathways. Their conclusion was based on the analysis of the effects from suppressing either one or both pathways on ginsenoside accumulation in *Panax ginseng* hairy roots with specific inhibitors (mevinolin and fosmidomycin).[42]

The biosynthetic pathway can be briefly outlined as follows

a. DMAPP is linked with IPP to form FPP, a process that is catalyzed by farnesyl diphosphate synthase (FPS).
b. FPP is linked head-to-tail with another FPP unit under the influence of squalene synthase (SS) to form squalene.
c. Squalene epoxidase catalyzes the epoxygenation of squalene to 2, 3-oxidosqualene.
d. 2, 3-oxidosqualene is cyclized under the catalysis of a family of enzymes called oxidosqualene cyclases to give products diverse in their basic backbone skeletons. Specifically, dammarenediol synthase (DS) catalyzes the synthesis of dammarenediol, forming tetracyclic skeletons for the *dammarane-type* of ginsenosides. β-amyrin synthase (βAS) is responsible for the synthesis of the intermediate, β-amyrin, which eventually leads to the formation of *oleanane-type* ginsenosides.
e. Finally, through the mediated actions of cytochrome P450s and glycosyltransferases, the intermediates, dammarenediol and β-amyrin are transformed into ginsenosides via hydroxylation and glycosylation reactions.

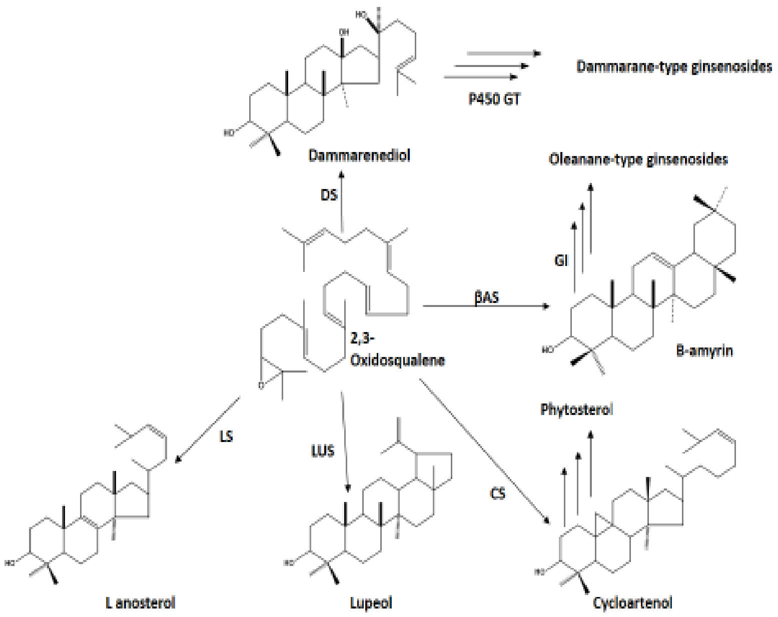

Fig. 2: Biosynthetic pathway of ginsenosides[41]

CONCLUSION

The elucidation of the various processes underlining the biosynthesis is still ongoing in respect of the various enzymes that catalyze the various steps in all saponin-producing plants. A knowledge of this biosynthetic pathway is very helpful in the cultivation of genuine ginseng breeds.

REFERENCES

1. Van Dyck, S. et al. Localization of secondary metabolites in marine invertebrates: contribution of MALDI MSI for the study of saponins in Cuvierian tubules of H. forskali. *PLoS One*, 2010; 5: e13923.
2. Augustin, J.M., Kuzina, V., Andersen, S.B. & Bak, S. Molecular activities, biosynthesis and evolution of triterpenoid saponins. *Phytochemistry*, 2011; 72: 435-57.
3. Sparg, S.G., Light, M.E. & van Staden, J. Biological activities and distribution of plant saponins. *J Ethnopharmacol*, 2004; 94: 219-43.
4. Szakiel, A., Pączkowski, C. & Henry, M. Influence of environmental abiotic factors on the content of saponins in plants. *Phytochemistry Reviews*., 2011; 10: 471-491.
5. Guclu-Ustundag, O. & Mazza, G. Saponins: properties, applications and processing. *Crit Rev Food Sci Nutr*, 2007; 47: 231-58.

6. Petit, P.R. et al. Steroid saponins from fenugreek seeds: extraction, purification, and pharmacological investigation on feeding behavior and plasma cholesterol. *Steroids*, 1995; 60, 674-80.
7. Price, K.R., Johnson, I.T. & Fenwick, G.R. The chemistry and biological significance of saponins in foods and feedingstuffs. *Crit Rev Food Sci Nutr*, 1987; 26: 27-135.
8. Abe, I., Rohmer, M. & Prestwich, G.D. Enzymatic cyclization of squalene and oxidosqualene to sterols and triterpenes. *Chemical Reviews*, 1993; 93: 2189-2206.
9. Haralampidis, K. et al. A new class of oxidosqualene cyclases directs synthesis of antimicrobial phytoprotectants in monocots. *Proc Natl Acad Sci U S A*, 2001; 98: 13431-6.
10. Kalinowska, M., Zimowski, J., Pączkowski, C. & Wojciechowski, Z.A. The Formation of Sugar Chains in Triterpenoid Saponins and Glycoalkaloids. *Phytochemistry Reviews*, 2005; 4: 237-257.
11. Yang, W.Z., Hu, Y., Wu, W.Y., Ye, M. & Guo, D.A. Saponins in the genus Panax L. (Araliaceae): a systematic review of their chemical diversity. *Phytochemistry*, 2014; 106: 7-24.
12. Shin, B.-K., Kwon, S.W. & Park, J.H. Chemical diversity of ginseng saponins from Panax ginseng. *Journal of Ginseng Research*, 2015; 39: 287-298.
13. Holstein, S.A. & Hohl, R.J. Isoprenoids: remarkable diversity of form and function. *Lipids*, 2004; 39: 293-309.
14. Phillips, D.R., Rasbery, J.M., Bartel, B. & Matsuda, S.P. Biosynthetic diversity in plant triterpene cyclization. *Curr Opin Plant Biol*., 2006; 9: 305-14.
15. Anna, C.A.Y. et al. Biosynthesis of Plant Triterpenoid Saponins: Genes, Enzymes and their Regulation. *Mini-Reviews in Organic Chemistry*, 2014; 11: 292-306.
16. Kolesnikova, M.D. et al. Stereochemistry of water addition in triterpene synthesis: the structure of arabidiol. *Org Lett*, 2007; 9: 2183-6.
17. Ryu, J.H., Park, J., Eun, J., Jung, J. & Sohn, D.H. A dammarane glycoside from Korean red ginseng. *Phytochemistry*, 1997; 44: 931-933.
18. Ma, W.G. et al. Saponins from the roots of Panax notoginseng. *Phytochemistry*, 1999; 52: 1133-1139.
19. Xiong, Q. et al. Structure and reactivity of the dammarenyl cation: configurational transmission in triterpene synthesis. *J Org Chem*., 2005; 70: 5362-75.
20. Cheng, Z.H., Yu, B.Y. & Yang, X.W. 27-Nor-triterpenoid glycosides from Mitragyna inermis. *Phytochemistry*, 2002; 61: 379-82.

21. Sahpaz, S., Gupta, M.P. & Hostettmann, K. Triterpene saponins from Randia formosa. *Phytochemistry*, 2000; 54: 77-84.
22. Sanoko, R., Speranza, G., Pizza, C. & de Tommasi, N. Triterpene saponins from Alternanthera repens. *Phytochemistry*, 1999; 51: 1043-1047.
23. Biswas, T., Gupta, M., Achari, B. & Pal, B.C. Hopane-type saponins from Glinus lotoides Linn. *Phytochemistry*, 2005; 66: 621-6.
24. Sahu, N.P., Koike, K., Banerjee, S., Achari, B. & Nikaido, T. Triterpenoid saponins from Mollugo spergula. *Phytochemistry*, 2001; 58: 1177-82.
25. Radwan, M.M., El-Sebakhy, N.A., Asaad, A.M., Toaima, S.M. & Kingston, D.G. Kahiricosides II-V, cycloartane glycosides from an Egyptian collection of Astragalus kahiricus. *Phytochemistry*, 2004; 65: 2909-13.
26. Verotta, L., Tato, M., El-Sebakhy, N.A. & Toaima, S.M. Cycloartane triterpene glycosides from Astragalus sieberi. *Phytochemistry*, 1998; 48: 1403-1409.
27. Verotta, L. et al. Cycloartane saponins from Astragalus peregrinus as modulators of lymphocyte proliferation. *Fitoterapia*, 2001; 72: 894-905.
28. Oobayashi, K., Yoshikawa, K. & Arihara, S. The International Journal of Plant BiochemistryStructural revision of bryonoside and structure elucidation of minor saponins from Bryonia dioica. *Phytochemistry*, 1992; 31: 943-946.
29. Vincken, J.P., Heng, L., de Groot, A. & Gruppen, H. Saponins, classification and occurrence in the plant kingdom. *Phytochemistry*, 2007; 68: 275-97.
30. Mamedova, R.P., Agzamova, M.A. & Isaev, M.I. Triterpene Glycosides of Astragalus and Their Genins. LXX. Orbicoside, the First Lanostane Glycoside from Astragalus Plants. *Chemistry of Natural Compounds*, 2003; 39: 583-585.
31. Corea, G., Fattorusso, E., Lanzotti, V., Capasso, R. & Izzo, A.A. Antispasmodic saponins from bulbs of red onion, Allium cepa L. var. Tropea. *J Agric Food Chem*., 2003; 53: 935-40.
32. Heng, L. et al. Stability of pea DDMP saponin and the mechanism of its decomposition. *Food Chemistry*, 2006; 99: 326-334.
33. Kennelly, E.J. et al. Novel Highly Sweet Secodammarane Glycosides from Pterocarya paliurus. *Journal of Agricultural and Food Chemistry*, 1995; 43: 2602-2607.
34. Oulad-Ali, A., Guillaume, D., Weniger, B., Jiang, Y. & Anton, R. The International Journal of Plant BiochemistryMabiosides C-E: triterpenoid saponins from the bark of Colubrina elliptica. *Phytochemistry*, 1994; 36: 445-448.

35. Lavaud, C. et al. Saponins from Steganotaenia araliacea. *Phytochemistry*, 1992; 31: 3177-81.
36. Jia, Z., Koike, K., Kudo, M., Li, H. & Nikaido, T. Triterpenoid saponins and sapogenins from Vaccaria segetalis. *Phytochemistry*, 1998; 48: 529-36.
37. Ueckert, J., Wray, V., Nimtz, M. & Schopke, T. Noroleanane saponins from Celmisia spectabilis. *Phytochemistry*, 1998; 49: 2487-92.
38. Park, S.Y., Chang, S.Y., Oh, O.J., Yook, C. & Nohara, T. nor-Oleanene type triterpene glycosides from the leaves of Acanthopanax japonicus. *Phytochemistry*, 2002; 59: 379-84.
39. Petersen, M. & Seitz, H.U. Reconstitution of cytochrome P-450-dependent digitoxin 12 beta-hydroxylase from cell cultures of foxglove (Digitalis lanata EHRH.). *Biochem J*., 1988; 252: 537-43.
40. Rohdich, F. et al. The deoxyxylulose phosphate pathway of isoprenoid biosynthesis: studies on the mechanisms of the reactions catalyzed by IspG and IspH protein. *Proc Natl Acad Sci U S A*, 2003; 100: 1586-91.
41. Liang, Y. & Zhao, S. Progress in understanding of ginsenoside biosynthesis. *Plant Biol (Stuttg),* 2008; 10: 415-21.
42. Zhao, S. et al. Both the mevalonate and the non-mevalonate pathways are involved in ginsenoside biosynthesis. *Plant Cell Rep*., 2014; 33: 393-400.