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Saponins in pharmaceutical applications and its extraction methods from ginseng and other medicinal herbs including *Codonopsis* species

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Abstract
Saponins extracted from ginsengs and medicinal plants including *Codonopsis* species have drawn huge research interests, especially in VietNam and Asian countries where traditional uses of herbs are common practices. Hence, finding proper extraction techniques for the recovery of saponins is extremely crucial for both research and industrial development. The objectives of this paper were to review: i) pharmaceutical applications of saponins and ii) current status and advancement in techniques for saponin extraction from ginsengs and herbs, including their strengths, limitations, and efficiency. The therapeutic properties of saponins are varied including the effects of anti-cancer, anti-central nervous system disorder, antimicrobial, anti-inflammatory, anti-obesity, anti-cardiovascular diseases, and anti-diabetic, etc., together with the enhancement in human health and sexuality. Current extraction methods for saponins can be divided into physical, chemical and biochemical methods. In general, recent green extractions are attracting attention regardless of their categories, mostly due to their effectiveness in saponin recovery with sustainable, non-hazardous and cost-effective advantages, especially the enzyme-assisted method for ginsengs and herbs extraction. This review contributes more insight into the status of saponin extraction from ginsengs and herbs, especially for upcoming research on new medicinal targets.

Keywords: *Codonopsis pilosula*, extraction, ginseng, saponin

Introduction

Over the years, numerous reports have documented adverse effects caused by synthetic drugs, leading to a growing demand for traditional and folk medicine. Bioactive medicinal plants, used for centuries as ingredients in folk medicine to treat a wide range of illnesses, have gained renewed interest. Among the various phytochemicals present in these herbs, saponins have recently become a focal point of study. Research has shown that saponin-rich plant extracts possess multiple properties including enhancement of energy and sexual function in men, anti-central nervous system disorder, antimicrobial, antidiabetic, anti-cardiovascular disease, etc. [1]. although the anti-obesity and anticancer activities of saponins are not fully understood, recent studies suggest their potential in treating these conditions.

Saponins, synthesized as secondary metabolites in a variety of plant species, are predominantly found in ginsengs and other medicinal herbs, e.g. *Codonopsis* species. Within plant cells, they accumulate as non-functional precursors. These compounds are derived from different plant parts, with their distribution varying significantly among plant organs, and they exist in different classes such as triterpenoid, steroid, and glycoalkaloids [2]. Over the past decade, extensive reviews by numerous research groups have focused on elucidating the structure, distribution, biosynthesis, commercial, and pharmacological importance of saponins, as well as identifying the most efficient extraction methods. Researchers from various scientific fields often face challenges in the initial extraction process before isolating and identifying specific saponins responsible for biological activities. Therefore, this review aims to provide a comprehensive overview of the beneficial effects of saponins on human health, along with a detailed review of the various extraction methods employed to isolate them, highlighting their principles, advantages, and limitations.

Saponins and its potential in pharmaceutical industry

1. Pharmaceutical activities of saponins

Saponins, a group of secondary metabolites, are generally found in the roots, leaves, fruits, flowers, and seeds in the plant kingdom and some marine species. In addition, saponins are divided into two major classes which are triterpenoid and steroid glycosides whose structure characterization are varied by the numbers of sugar units attached at different positions. Their amphiphilic character, which arises from the combination of hydrophilic sugar chains and a lipophilic sapogenin, allows them to reduce surface tension and create foam when shaken in aqueous solution. And the absorption of ginseng saponins is quite low when they are administered orally due to their low membrane permeability and they are extensively metabolized in the gastrointestinal tract [3]. Saponins are employed in both traditional and modern pharmaceutical applications, and it has been reported to have therapeutic potential in hypertension, stress, and different neurological disorders such as Alzheimer's disease (AD), Parkinson disease (PD), and Huntington disease [4].

1.1. Anti-cancer effect

Therapeutic effects of saponins on cancer cells has recently gained huge research interest in the pharmaceutical sector. Studies have consistently reported that ginsenosides have anti-cancer effects in several cancer models, even though the exact anticancer mechanism has not been elucidated [5]. These ginsenosides have possessed outstanding potential in inhibition of cancer cells under *in vitro* and *in vivo* conditions [6]:

i) Rg3, Rg5, and Rk1 with anticancer properties,
ii) Rd and Rh2 with inhibitory capability of tumor migration and metastasis inhibition in liver cancer; or
iii) Rg3, an anti-cancer drug, with the suppression capability of tumor growth and angiogenesis in endothelial progenitor cell bioactivities.

1.2. Anti-central nervous system disorder effect

Ginsenosides (Re, Rg1, Rg3, Rb1, Rb2) and notoginsenoside (R1) are reported in a variety of *in vitro* and *in vivo* studies to reduce the amyloid b peptide concentration, which in turn protects against AD [7]. Ginsenosides Rb1, Rg1, and Rd are shown to exert neuroprotective capability by the inhibition of oxidative stress and neuroinflammation. These ginsenosides also decrease toxin-induced apoptosis. Ginsenoside Rg3 also enhanced learning and memory im- pairments in lipopolysaccharide-induced cognitively impaired mice. Using ginseng root daily for 12 weeks did improve mental performance significantly as revealed by a clinical study conducted among patients with AD in the Department of Neurology at the Clinical Research Institute in South Korea [8].

1.3. Enhancing of energy and sexuality

Sexual dysfunction treatment using ginseng and medicinal herbs has been widely applied in Asia. Recent research on laboratory animals has indicated that both Asian and American ginseng improve libido and copulatory performance. Ginsenoside Rg1 could be a promising new drug for erectile dysfunction and low libido. And two pairs of geometric isomers, i.e. Rk1/Rg5 and Rk3/Rh4, isolated from steamed ginseng, were determined to play an important role in the treatment of erectile dysfunction [9]. A recent study conducted with 587 men (20 to 70 years old) with mild to moderate erectile dysfunction concluded that ginseng may improve men's ability to have intercourse, and might have little to no adverse effect on adverse [10].

1.4. Antimicrobial effect

A number of studies have been conducted to evaluate the antiviral and antimicrobial activity of ginseng extract and its components. Ginsenosides Rg1, Re, Rf, Rh1, Rg2(s), Rg2(r), Rb1, Rc, Rb2, Rd, Rg3(s), and Rg3 stimulated the antiviral cytokines IFN-g and IFN-a in response to H5N1 influenza virus challenge [11]. Additionally, acting as a bactericidal agent, ginseng strengthened resistance to experimental sepsis caused by *E. coli*. For instance, ginseng saponins together with kanamycin and cefotaxime were reported to successfully disrupt the cell membrane of *Staphylococcus aureus*, thereby decreasing infection [12].

1.5. Anti-inflammatory effect

Numerous *in vitro*, *in vivo*, and clinical investigations have revealed that *Panax notoginseng* saponins (PNS) may have anti-inflammatory properties [1]. In addition, ginsenoside Rc can inhibit the expression of macrophage-derived cytokines. Moreover, PNS modulated the proliferation and differentiation of Th17 cells by globally downregulating the expression of inflammatory cytokines and cell cycle genes. Hence, it could be potentially applied as an anti-inflammatory agent [13].

1.6. Anti-obesity effect

The processes by which ginseng prevents obesity have not been clearly elucidated. However, it also has been reported that ginseng has an anti-obesogenic effect [14]. *In vitro* and *in vivo* studies have suggested that ginsenosides have the potential to increase energy expenditure by stimulating the adenosine monophosphate activated kinase pathway and are capable of reducing energy intake in a similar way [15]. In addition, ginsenoside Rg1 suppresses early-stage differentiation via the activation of CHOP10 and attenuates fat accumulation *in vivo*, which indicates that Rg1 might have the potential to reduce body fat accumulation in the early stage of obesity.

1.7. Anti-cardiovascular disease effect

Active components of ginseng can stimulate nitric oxide production, inhibit reactive oxygen species production, increase blood circulation, and help in adjusting lipid profiles. Studies showed that *P. ginseng* can help maintain proper blood circulation and can boost vascular endothelial cell-derived nitric oxide secretion, which decreases blood pressure [16]. Other research also reported that some saponins have an effect on the coagulation cascade, including prothrombin and other coagulation factors [2].

1.8. Anti-diabetic effect

Hypoglycemic activity of most antidiabetic medicinal herbs has been attributed to the presence of saponins. And dietary saponins possessed the multidirectional anti-diabetic capabilities by concurrent regulation of various signaling pathways [17]. In addition, saponins have potential therapeutic benefits and are theorized as an alternative medication in decreasing serum blood glucose levels in the patient suffering from diabetes [18].

2. Current status of saponin extraction from ginsengs and other medicinal herbs including *Codonopsis* species

Codonopsis is a perennial plant genus in the *Campanulaceae* family with tuberous roots and twining stems. There are approximately 42 species which are mainly located in Asia countries (Korea, China, Thailand, Japan, and VietNam). *Codonopsis pilosula* and *Codonopsis javanica* are considered as a precious herb with high medical value and are the common species of *Codonopsis* spp. in VietNam. They are widely distributed from the northern region to the south-central provinces such as Lai Châu, Lào Cai, Hà Giang, Hòa Bình, Kon Tum, and Lâm Đồng. Their roots are important traditional medicines with a variety of therapeutic properties due to its composition, e.g. saponins, polysaccharides, polyacetylenes, polyenes, flavonoids, lignans, alkaloids, coumarins, terpenoids, steroids, organic acids, etc. [19].

Recently, the discovery of several therapeutic properties of saponins, especially anticancer activity, have strengthened the demand of seeking and extracting of saponins from medicinal plants. These have only driven the emergence of not only various new extraction technologies with the main purpose of maximizing the yield in order to accommodate the recent need but also other rich saponin botanicals such as *C. javanica* and *C. pilosula* besides ginsengs. Because saponins are recently one of the most interesting research subjects due to their potential for industrial processes, proper choices of extraction methods is essential [20].

The recent advances in extraction of bioactive compounds from plant materials have been intensively reviewed and this might be due to the recent improvement in public awareness of health care in both terms of prevention and treatment. Hence, the promotion in daily consumption of herbal extract is also high, leading to the need of effective extraction techniques [21]. In summary, methods for saponin extraction can be categorized into two groups, the conventional and the green technologies. The conventional extraction techniques are maceration, Soxhlet, and reflux extraction, where the green technologies are ultrasound-assisted, microwave-assisted, and accelerated solvent extraction [22]. The conventional extractions are based on the solubility of solute from herb materials into solvent. As a consequence, it often utilizes a significant amount of solvent even with the aid of elevated temperature by heating, and mechanical stirring or shaking. In contrast, green extractions employed much less hazardous chemicals, together with the advantage in energy efficiency using renewable feedstock, and pollution prevention [21]. Consequently, ethanol and water is usually used as extraction solvent by manipulating the extraction system pressure and temperature, as in pressurized liquid extraction.

Extraction methods for saponins from ginsengs and other medicinal herbs including *Codonopsis* species

1. Physical extraction techniques

1.1. Maceration

Maceration is a solid-liquid extraction for retrieving the bioactive compounds inside plant materials by immersing them in a particular solvent for a certain amount of time [23]. It is a very simple extraction with no complicated utensils and equipment for the setting of the extraction system, which has made it a popular choice for both research and industrial sectors worldwide. Although this method has a low extraction efficiency and a long extraction time that needs to be aided with mechanical shaker or magnetic stirring, it is very suitable for thermolabile plant material. Maceration managed to bring sufficient extraction yield of saponins from Korean ginseng (3.79%) [24] or *C. pilosula* (7.3%) [25]. Although both ethanol and methanol are common solvents used to extract saponins from herbal plants, ethanol is usually preferred probably due to its environment friendly characteristics. The only paramount factor to be paid attention in enhancing extractability is the understanding of similarity of bioactive compound interest and solvent polarity [20].

1.2. Supercritical fluid extracion

Supercritical fluid extraction (SFE) has been used to isolate natural products since the end of the 1970s, but only on a few products. Recently, SFE has been used with more attention to extract bioactive compounds from ginsengs and herbal plants, including Korean ginseng and American ginseng. in order to characterize compounds responsible for a specific functional activity. Compared to other conventional liquid extraction, SFE offers significant reduction in extraction time and organic solvent consumption [26]. However, using pure carbon dioxide (CO_2) for extraction and fractionation has a disadvantage which is no pure dipole moment, and CO_2 is an ineffective solvent for highly polar materials. Therefore, modifiers (ethanol, methanol, or n-hexane) could be used to increase the polarity of the fluid phase during extraction. When ginseng was dissolved in methanol, the total ginsenoside concentration was 71.8 ± 1.8 $mg.g^{-1}$, which was greater than that attained by being dissolved in dimethylsulfoxide (DMSO) (50.8 ± 2.1 $mg.g^{-1}$) [27].

1.3. Pulsed electric field extraction

Pulsed electric field extraction (PEFE) is known as an advanced non-thermal technique. PEFE uses electric pulses to induce local instability and tension in the cell membrane and destroy microbial and plant cells by creating pores in the membrane. And the food industry has paid more attention to this method as a nonthermal alternative to pasteurization in the past few years. In addition, this method has been used for the successful separation, intensification, stabilization, and dehydration of important compounds without affecting the nutritive properties [28]. Moreover, PEFE can be considered as the ideal technology of high efficiency and low temperature for natural ingredients extraction [29]. When conducting saponin extraction from *Panax ginseng* by PEFE method, the yield of the ginsenoside was significantly higher than the other methods including microwave-assisted extraction, heat reflux extraction, accelerated solvent extraction, and ultrasonic-assisted extraction. Because of this, the PEFE method has been considered as a promising and constructive method to extract ginsenosides. Besides, under the optimal conditions, the extraction speed of PEFE combined with commercial enzyme, which was β-glucosidase, was much faster, and the yield of total saponin was higher compared with traditional methods [28].

1.4. Microwave-assisted extraction

Microwave-assisted extraction (MAE) is an automated green technique to extract valuable products that was first appeared in 1995, and recently can be combined with microwave and traditional solvent extraction by using microwave energy to heat the sample solvent mixture for the isolation of analytes from a sample matrix [30]. Compared to other conventional methods, MAE has many advangtages such as shorter extraction time, less solvent, higher extraction rate and lower cost or even the possibility of simultaneously extracting multiple samples. It significantly reduced the extraction time from 12 hours to a few seconds, suggesting that it can be an alternative technique to the time-consuming conventional reflux method, together with increasing the ginsenoside yield in *P. ginseng* in comparison with Soxhlet extraction [31]. However, the total saponin content (TPC) extracted from Ngoc Linh ginseng (*Panax vietnamensis*) by microwave was much lower than that by using ultrasound and enzyme methods [32].

1.5. Ultrasonic-assisted extraction

Ultrasound-assisted extraction (UAE) uses ultrasound energy and solvents to extract target compounds from various plant matrices. Ultrasound is composed of mechanical sound waves originating from molecular movements that oscillate in a propagation medium. This method can be carried out at lower temperature which may prevent the thermal degradation of unstable ingredients in plant materials. Moreover, the extraction rate of the ultrasound-assisted processes was about three times faster than that of the traditional methods [33]. It has been noted that the yields of saponin and total extract with ultrasonic irradiation are larger than those without ultrasonic irradiation, and the yield of saponin increased by about 30% and the yield of whole extract by about 15% at an acoustic pressure of 67 kPa. It was also found that ultrasound significantly enhanced supercritical CO_2 reverse microemulsion extraction; hence, making it have more potential to be applied in the field of extraction. With the same ginseng root, the UAE produced much higher yield of TPC than MAE method. Under the UAE condition of 60°C for 2 hours, TPC yield achieved a maximum of 27.53 $mg.g^{-1}$. Its content was 1.40 times higher than MAE1 treatment for 6 min, 1.16 times higher than MAE2 treatment for 8 min [34].

2. Chemical extraction techniques

2.1. Reflux extraction

Reflux extraction is a traditional extraction method using only a flask and cooling above. The process based on the solubility of solute in the solvent, involves heating a solution to boiling and then returning the condensed vapors to the original flask. With this method, it is possible to extract the solid at a high temperature without losing solvent by evaporation. The system is widely used in herbal industries as it is more efficient than percolation or maceration and requires less extraction time and solvent but cannot be used for the extraction of thermolabile natural products [35]. TPC extracted from ginseng varied by numerous factors including extraction time, ethanol concentration, temperature and times of extraction. 70% ethanol was reported to be the optimal solvent for extraction of ginsenosides from ginseng powder. Besides, the amount of ginsenoside in the extract noticeably increased after 6 hours when the temperature was 80°C. As the ethanol concentration decreased, saponin yields first increased and then decreased. A longer extraction time led to higher yields of the ginsenosides Rb1 and Rd. The TPC’s purity increased as the ethanol concentration increased. The optimum conditions to maximize the yield, content, and antioxidant activity for saponins extracted from steamed *P. notoginseng* were obtained with extraction time of 1.51 hours and ethanol concentration of 60% [36].

2.2. Soxhlet extraction

Percolation and maceration techniques are combined in the Soxhlet extraction process. The extraction is conducted in a special apparatus known as Soxhlet apparatus designed by Franz von Soxhlet in 1879. Since then, the standard Soxhlet technique has been routinely applied in almost every analytical laboratory and remains the standard technique to which the performance of modern extraction techniques is compared [37]. Its most noticeable benefit is to keep the sample in contact with the new solvent segment, preventing the solvent from getting saturated with extractable material and thus enhances the removal of the analytes from the matrix. In contrast, the disadvantage is that the solute is always at the boiling temperature of the solvent, which may cause damage to thermolabile compounds. Another considerable drawback is that this technique is both time and solvent consuming. Additionally, the most common extractors use quite large amounts of purified solvent [38]. Different extraction times contributed to the amount of ginsenoside contents extracted from fresh *P. ginseng*. The TPC of ginseng increased proportionally with increasing extraction time (1, 2, and 8 hours). And the total ginsenoside content extracted from fresh American ginseng (8.0%) was greater than that extracted from steamed material (5.9%) [39].

2.3. Pressurized liquid extraction

Pressurized liquid extraction (PLE) is considered as a new and innovative technique used to extract samples with relatively small amounts of conventional solvent under high temperature and pressure. This method could have numerous benefits, including significant time and solvent savings and comparable or greater analyte extraction yields. Moreover, most of the instruments used were operated automated, allowing the development of less-labor methods and improving producibility [40]. However, the drawback of this very efficient process, particularly for complex biological matrices, is that the extracts are typically very dirty and require further purification. PLE has also been used to extract ginsenosides from *P. ginseng* and *Panax quinquefolius* for multiple targets. When compared with Soxhlet extraction, the total amount of saponin extracted by PLE was considerably higher while the time and volume of solvents required were lower. Besides, using aqueous non-ionic surfactant solutions as a solvent system in PLE could help the extraction be carried out at lower temperatures without a significant compromise in extraction efficiency, and thus avoiding the degradation of thermally unstable ingredients in plant materials. And the chemical characteristics were obviously diverse in different parts of *P. notoginseng*, which is beneficial for ginseng's pharmacological assessment and quality control. Meanwhile, the combined method of PLE and high performance liquid chromatography (HPLC) for the analysis of compounds in traditional medicines and found that higher amounts of leaf ginsenosides corresponded to lower amounts of root [1].

3. Biochemical method: Enzyme-assisted extraction

Various green extraction strategies have been figured out so as to maximize extraction efficiency of active ingredients from different parts of ginsengs and medicinal herbs. A wide range of conventional solvent based extraction methods are predominantly being used in the food sector for isolation of pharmaceutically important compounds. Apart from soluble compounds within the plant cell wall, there are certain compounds bound to the cell wall, preventing them from leaching out when using conventional methods by aqueous solvents. Besides, the extraction efficiency of conventional extraction techniques can be reduced by the presence of various polysaccharides such as hemicelluloses, starch, pectin in large amounts inside the cell wall [41]. Hence, conventional extraction techniques experience low extraction yields, time inefficiency and inferior extract quality due to unavoidable presence of traces of organic solvents in them. Therefore, to extract the phytochemicals from hard plant materials, it necessitates using the most suitable and cutting-edge extraction technique.

Recently, using enzymes as a catalyst to extract bioactive ingredients from plants has potentially been used as an alternative to conventional solvent extraction methods and become more popular because of being a sustainable, efficient, and eco-friendly extraction technology [42]. The basic principle of enzyme assisted extraction is the disruption of biological barriers, namely cell walls and membranes, by hydrolysing it using enzyme as a catalyst under optimum experimental conditions, in order to release the intracellular components. The plant cell wall binds to the active site of the enzyme, causing the enzyme’s shape so that the substrate fits into its active site, thus optimizing the interaction between the two. Change in the shape of enzyme leads to breakage of bonds of the cell wall, thereby liberating the active constituents out of it. Therefore, this enzyme-assisted green extraction method offers certain benefits such as reductions in hazardous solvents and extraction time. Additionally, bioactive compounds which are sensitive to heat such as flavours, pigments, oil, etc can be extracted easily due to controlled temperature conditions. A variety of carbohydrate hydrolysing enzymes is generally utilized during enzyme assisted extraction [43].

Cellulase has been employed to extract bioactive ingredients from plant sources such as polysaccharides from *Malva sylvestris* and saponins from *Gomphrena celosioides* Mart. Furthermore, optimal extraction conditions were obtained when adding 1% α-amylase at 90°C in 3.5 hours. The obtained *C. javanica*'s extract showed significant higher levels of TPC and antioxidant capacity compared to ones without enzymes [42]. In addition, cellulase-assisted extraction in *C. javanica* with optimal conditions (material:solvent ratio, enzyme:subtrate ratio, extraction duration, and temperature) significantly doubled the amount of TPC compared with the control [44]. Recently, the combination of different enzymes (cellulase, pectinase, amylase, maltase, and flavor protease) to extract active ingredients from American ginseng showed to be a promising method in improving the yield of important nutrients and functional compounds [45].

Conclusion

In conclusion, saponins, a diverse class of glycosides found in ginsengs, hold significant promise in pharmaceutical applications due to their unique biological properties. These compounds exhibit a range of bioactivities, including anti-inflammatory, immunomodulatory, and anticancer effects, etc. making them valuable in drug development and therapeutic interventions.

Extraction methods used to isolate saponins from ginsengs have attracted much attention from numerous researchers recently. This review updates and provides information of different extraction techniques, including physical extractions, chemical extractions, and biochemical extractions. Each method presents its own set of advantages and limitations, making the choice of technique dependent on the specific goals of the extraction process. Ultimately, the selection of an appropriate saponin extraction method should align with the desired purity, efficiency, and economic feasibility to achieve optimal results.

The saponin extraction process is crucial for leveraging *C. pilosula*'s therapeutic potential, including its immune-boosting and anti-inflammatory properties. Future studies should aim to refine these methods further, not only optimizing the yield of ginsenosides but also improving scalability for industrial applications.

Competing Interests

The authors declare no conflicts of interest.

Author Contributions

Phu H. Le conceived the idea, provided support, and critically revised the manuscript. Phuc N.T. Le structured the contents. Phuc N.T. Le and Anh N. Nguyen wrote the manuscript. An D.X. Nguyen contributed to the finding of materials. An D.X. Nguyen and Anh T.N. Nguyen contributed to proofreading. All authors read and approved the final manuscript.

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