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MINI-REVIEW

Quality, safety and efficacy profiling of ginseng adventitious roots produced in vitro

Hosakatte Niranjana Murthy[1,2] · Vijayalaxmi S. Dandin[2] · So-Young Park[2] · Kee-Yoeup Paek[2]

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Abstract

Ginseng (*Panax ginseng* C. A. Meyer, Family Araliaceae) is one of the major medicinal and nutraceutical plants, which is native to oriental region. It is used worldwide as a popular herbal medicine because of its pharmacological effects like anti-oxidative, anti aging, anti-cancer, adaptogenic, and other health-improving activities. Chief components of ginseng identified till date are ginsenosides, a group of saponins with triterpenoid structure. Ginseng is cultivated under controlled conditions, and for harvesting of fully grown roots of the plant, the cultivation takes long duration of about 5–7 years and cultivated ginseng roots are inferior in quality and ginsenoside content. Wild Mountain ginseng is superior in quality and ginsenoside content but is scarce in nature. Therefore, for obtaining the useful compounds of this plant at commercial scale, cell and organ cultures especially adventitious roots have been established by using superior clones of wild mountain ginseng, ginseng biomass is produced by applying large scale bioreactors. In this paper, an effort has been made to shed light on the scientific literature and to decipher the evidences for quality, safety, and efficacy of ginseng adventitious roots produced from in vitro cultures.

Keywords Adventitious roots · Efficacy · Ginseng · Safety · Tissue culture · Quality

Introduction

Ginseng (Botanical name: *Panax ginseng* C. A. Meyer; Common name: 'Korean ginseng or Asian ginseng; Family: Araliaceae) is a popular medicinal plant which has been used for thousands of years in Russia, China, Korea and Japan. The word 'Panax', first used by Russian botanist Carl Anton Von Meyer is derived from Greek word meaning 'all healing' (Court 2000). On the other hand, the English word ginseng is derived from the Chinese term 'renshen' which literally means 'man-root' (roots look like human body). Ginseng has been used in Traditional Chinese Medicine and Oriental Medicine to revitalize the body and mind, to increase physical strength, prevent aging, and increase vigor (Choi 2008). Ginseng has its pharmacological action on improved brain function, pain-relief, anti-tumor activity, enhanced immune function, anti-diabetic effects, enhanced liver function, blood pressure control, anti-fatigue and anti-stress effects, and improved sexual functions, anti-oxidative and anti-aging effects (Choi 2008; Park et al. 2005). *Panax ginseng* has been used in therapeutics since ancient times in herbal medicine. Currently, *Panax ginseng* is popular worldwide as an invaluable nutraceutical and medicinal plant (Neale et al. 2012).

Chemical profile of *Panax ginseng* chiefly depicts triterpenoid glycosides, or saponins, commonly referred to as 'ginsenosides' along with other active ingredients like amino acids, alkaloids, phenols, proteins, polypeptides, polysaccharides, fatty acids, and vitamin B1 and B2 in abundance in different parts of the plant (Blumenthal 2003). Ginsenosides are grouped into three categories based on their structure namely Rb group (protopanaxadiols), the Rg groups (protopanaxatriols), and Ro group (Oleanolic acid) (Park et al. 2005). The ginseng roots which are found in commercial markets are of two types namely 'red ginseng' and 'white ginseng'. 'Red ginseng' is prepared normally by steaming and drying the ginseng roots, while the peeled roots dried without steaming are known as 'white ginseng'. It was reported that certain ginsenosides undergo deglycosylation during streaming and converted to other forms (Park et al. 2005).

✉ Hosakatte Niranjana Murthy
nmurthy60@yahoo.co.in

[1] Department of Botany, Karnatak University, Dharwad 580003, India

[2] Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Cheongju 261-763, Republic of Korea

Therefore, some ginsenosides are common in red and white ginseng, whereas some others are unique to either white ginseng or red ginseng. Thirty-eight ginsenosides are isolated from Korean ginseng roots; among these, 18 are common in both red and white ginseng namely ginsenoside-R_0, -Ra, -Ra_2, -Ra_3, -Rb_1, -Rb_2, -Rb_3, -Rc, -Rd, -Re, -Rf, -Rg_1, -Rg_2, -Rg_3, -Rh, quinquenoside-R_3, notoginsenoside R_1, 20-gulco-ginsenoside-Rf, whereas malonyl-ginsenoside-Rb_1, -Rb_2, -Rc,-Rd, koryoginsenoside-R_1, koryoginsenoside-R_2, polyacyleneginsenoside-R_0 are unique to white ginseng. Ginsenoside-Rh_2, -Rs_1, -Rs_2, -Rs_3, ginsenoside Rf_2, 20(S)-ginsenoside-Rg_3, 20(S)-ginsenoside-Rg_2, 20(S)-ginsenoside-Rh_1, notoginsenoside R_4, ginsenoside Rh_4, ginsenoside-Rg_5, Rg_6, 20(E)-ginsenoside-F_4 are reported to be found in only red ginseng (Choi 2008). Each ginsenoside possesses different pharmacological actions, and Korean ginseng was reported to influence better pharmacological efficacy compared to American ginseng (*Panax quinquefolius*) and Sanchi ginseng (Chinese ginseng; *Panax notoginseng*) (Choi 2008).

Korean ginseng which occurs naturally in its original habitat is rare and is a highly expensive commodity. Ginseng is cultivated in the fields in Korea, Japan, and China, and it takes 5–7 years for its cultivation to harvest the fully grown mature roots. Quality control management of ginseng cultivation is a laborious task as it is affected by environmental factors, such as soil, climate, shade, pathogens, and pests. To overcome these shortcomings, cell, tissue, and organ cultures have been effectively explored for more rapid and higher production of ginsenosides in Korean ginseng (Murthy et al. 2014a; Paek et al. 2009; Thanh et al. 2014a, b; Wu and Zhong 1999). Plant cell, tissue, and organ cultures offer a number of advantages over the conventional use of plants as sources of phytochemicals by exclusively being independent of geographical, seasonal, and environmental variations, by being reliable in continuous production of uniform quality and yield, in preventing the use of pesticide and herbicide applications and in having comparatively short growth cycles (Rao and Ravishankar 2002; Wilson and Roberts 2012). In many cases, the formation of desired products in plant cell, tissue, and organ cultures can be emphatically enhanced by altering the different key factors including the ionic strength of the basal medium, phosphate and nitrate levels, the level and the type sugars, and growth regulators (Murthy et al. 2014b; Rao and Ravishankar 2002). Additionally, a number of physical factors such as inoculum density, temperature, light quality and intensity, hydrogen ion concentration (pH) of nutrient medium, agitation and aeration of the batch cultures can be modulated to increase the production of desired product (Murthy et al. 2014b). Elicitation and precursor feeding and removal of the products from cultured cells and medium are the further approaches, and these strategies have been effectively applied to improve the accumulation of secondary metabolites in plant cell and organ cultures (Zhao et al. 2005; Murthy et al. 2014b).

Generally, the productivity of a plant in vitro culture is dependent on the degree of differentiation; in many cases, meager amount of desired secondary products is accumulated in cell cultures (Kolewe et al. 2008). Therefore, adventitious root, hairy root, embryo, protocorm, and shoot cultures have been explored as alternatives for the production of secondary metabolites in many medicinal plants (Cui et al. 2014; Georgiev et al. 2012; Murthy et al. 2008, 2016; Shohael et al. 2014). Extensive research efforts have been made for the production of ginsenosides from Korean ginseng through cell cultures, and major factors which affect biomass and metabolite accumulation have been systematically assessed (Wu and Zhong 1999). More recently, adventitious root cultures have been induced in *Panax ginseng*, and factors affecting biomass and ginsenoside accumulation have been standardized. Even bioreactor and bioprocess technologies have been skillfully engineered for the production of ginsenosides from adventitious root cultures (Murthy et al. 2014a, 2016). *Panax ginseng* biomass and ginsenosides are produced from cell and adventitious root cultures using bioreactor technology by Nitto Denko Corporation, Osaka, Japan; Unhaw Biotech Corporation, Jeonbuk, South Korea; CBN Biotech Company, Ochang-eup, South Korea; Bio-FD&C Co. Ltd., Smart Valley A-dong, South Korea. The ginseng biomass produced from bioreactor cultures is used commercially for the production of ginseng extract, tablets, dietary supplements, cosmetics, and other pharmaceutical and health-related products (Murthy et al. 2014a). Substantial research has been carried on biosafety, toxicology, efficacy of ginseng biomass produced through cell, tissue and organ cultures. In this review, we update the research progress made on the quality, safety, and efficacy profiling of ginseng adventitious roots produced in vitro.

Profiling of ginseng adventitious root biomass quality and biosafety of in vitro products produced

Initiation and proliferation of cultures—current technology

In order to obtain quality products, selection of suitable plant material, i.e., selection of superior genotype and responsive plant parts used for the initiation of in vitro culture, is necessary. Furthermore, methods used for the production of raw material viz. selection of suitable explants for induction of cell lines or adventitious or hairy root lines need to be optimized. Once cell lines are established, selection of cell or organ lines (clones) for the production of biomass needs to be done and further optimization of in vitro conditions viz. medium, ionic strength, growth regulator and its concentration, medium pH, temperature, light intensity/quality will be necessary.

Selection of suitable bioreactors, agitation, aeration, mode of operation, and bioreactor conditions will be needed for large-scale production (Murthy et al. 2014a, 2016).

Hundred year old mountain ginseng had been selected as quality explants with potential to produce quality and quantity of ginsenoside in vitro (Table 1). Cell, callus, hairy root, and adventitious root cultures were established (Lee et al. 2018; Paek et al. 2009; Murthy et al. 2017b). Among the various types of cultures viz. cell, callus, adventitious roots, hairy roots, it was a specific clone of adventitious root cultures (AR-4) that possessed the highest content of ginsenosides (Table 1). Diverse parameters affecting biomass and metabolite production such as ionic strength of the medium, phosphate and nitrate levels, the level of the sugars, and growth regulators, inoculum density, temperature, light quality and intensity, hydrogen ion concentration (pH) of nutrient medium, agitation and aeration of the batch cultures have been systematically worked out by various investigators for ginseng adventitious root cultures (Murthy et al. 2014a). Modified Murashige and Skoog medium (without ammonium nitrate) supplemented with 19.68 μM indole butyric acid and 3% sucrose and incubation of cultures in continuous dark at a constant ambient temperature of 20 °C was suitable for biomass and metabolite accumulation. Similarly, 5 g/l inoculum, aeration of cultures with 0.1 vvm (air volume/medium volume/min) sterile air enriched oxygen (40%) enhanced the accumulation of biomass and metabolites in modified airlift bioreactor cultures (Paek et al. 2009). Cultivation of adventitious roots for 40 days in optimized conditions and subsequent elicitation of cultures with methyl jamonate (100 μM) for subsequent 10 days led to 7-fold accumulation of ginsenosides compared to un-elicitated cultures (Kim et al. 2004). Various strategies such as precursor or fresh medium feeding have been also tested and in ginseng adventitious root cultures; a medium replenishment (medium exchange) method gave in an increased ginseng biomass and ginsenosides (Jeong et al. 2008).

Safety consideration for processing of ginseng adventitious roots

CBN Biotech Company, South Korea, is operating 10,000-L commercial airlift bioreactors for the production of *Panax ginseng* adventitious root biomass and producing ~45 t of ginseng adventitious root biomass every year and root biomass is utilized by pharmaceutical, food, and cosmetic industries. Preservation of adventitious roots is essential to avoid microbial contamination and to prevent loss of bioactive compounds. Various methods such as far-infrared drying, freeze drying, airflow drying have been tested for drying the ginseng adventitious roots, and it was found that forced air drying was superior to other two methods (Kim et al. 2008). Extraction of bioactive ingredients from raw material is another crucial procedure during which there can be decomposition of target compounds and sometimes if method is inferior, it ends with accumulation of toxic compounds (Azmir et al. 2013). Extraction of bioactive ingredients from powdered ginseng adventitious roots has been worked by Kim et al. (2007) and they have compared heat reflux extraction method in comparison with ultrasonic and microwave extraction. They have also tested four variables such as type, concentration of solvent (water, 10, 30, 50, 70, and 100% ethanol), extraction temperature (40, 60, and 80 °C), and duration (2, 4, 6, and 8 h). Isolation of bioactive compounds by heat reflux extraction and treatment of samples with 70% ethanol at 80 °C for 6 h was found to be suitable for extraction of ginsenosides along with total phenolics and polysaccharides from adventitious roots of ginseng.

Table 1 Accumulation of ginsenosides in different ginseng cultures in comparison with natural roots

Ginseng	Ginsenosides (mg/g DW)			
	Protopanaxatriol (D)	Protopanaxadiol (T)	D/T	Total
Cultivated ginseng roots	5.25 ± 0.15	6.10 ± 0.40	1.16	11.35 ± 0.55
Red ginseng roots	4.94 ± 0.20	9.09 ± 0.90	1.83	14.04 ± 1.09
Mountain ginseng roots	6.61 ± 0.34	7.57 ± 0.24	1.10	14.19 ± 1.20
Adventitious root cultures (line # AR-1)[a,b]	2.75 ± 0.70	1.86 ± 0.30	0.73	4.61 ± 0.98
Adventitious root cultures (line # AR-2)[a,b]	3.41 ± 0.00	2.57 ± 0.04	0.75	5.98 ± 0.04
Adventitious root cultures (line # AR-3)[a,b]	3.34 ± 0.80	14.74 ± 1.05	4.41	18.09 ± 1.03
Adventitious root cultures (line # AR-4)[a,b]	2.81 ± 0.33	29.66 ± 2.30	10.56	32.46 ± 2.28
Callus cultures[a,b]	0.31 ± 0.04	2.54 ± 0.31	8.45	2.85 ± 0.26
Hairy root cultures[a,b]	3.56 ± 0.22	6.26 ± 0.41	1.75	9.83 ± 1.05
Cell suspension cultures[a,b]	2.65 ± 0.02	6.17 ± 0.26	2.32	7.86 ± 0.06

[a] Callus, hairy roots, and adventitious roots induced from roots of wild Korean ginseng

[b] Amount of ginsenosides quantified in cell, hairy root, adventitious root biomass using high pressure liquid chromatography

Profiling of ginseng adventitious root biomass quality

The quality and consistency of the bioactive ingredients in tissue-cultured products require identity and purity of phytochemicals. Further, the raw material should be free from microbial contamination, toxins, and impurities. Therefore, biological, chemical, and nutritional analyses have been recommended for plant-, cell-, and tissue-cultured products (Murthy et al. 2015, 2017a). The chemical analysis of ginseng adventitious roots revealed that biomass was rich in ginsenosides (Table 2, Murthy et al. 2014c, d, e). Additionally, ginseng adventitious roots also contained carbohydrate, protein, vitamin, and mineral nutrients and free from microbial contaminants, heavy metals, and other contaminants (Table 3).

Profiling of safety of ginseng adventitious root biomass produced in vitro

Safety evaluation of plant cell-, tissue-, and organ-cultured raw material involves (a) in vitro toxicological evaluation with bacteria and mammalian cell lines to assess point and chromosomal mutations; (b) in vivo toxicological evaluation with rodents and non-rodents to assess mutagenicity, carcinogenicity, developmental toxicity, reproductive toxicity, immunotoxicity, and neurotoxicity; (c) clinical studies with humans using placebo-controlled groups (Murthy et al. 2014e). The mutagenicity, genotoxicity, chromosomal aberration, and micronucleus tests were conducted by Biotoxtech, South Korea (a Korean Food and Drug administration approved laboratory). In the mutagenicity test (Ames test) of ginseng adventitious root powder (312.5, 625, 1250, 2500, and 5000 μg/plate), using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and the *Escherichia coli* strain WP2*uvr*A^{-} with or without S9 mixture, the results showed very few mutations which were lesser than positive controls (2-aminoflurene, sodium azide, 9-aminoanthracene, Table 4). In vitro chromosomal aberration test with Chinese Hamster Lung (CHL) cultured cells in both a non-activated and an activated system, with or without a S9 mixture, for 6 h, and also non-activated system without a S9 mixture for 24 h, at doses of 150, 300, and 600 μg/ml, and results did not reveal any abnormalities, associated with the ginseng adventitious root powder doses up to 600 μg/ml (Table 5). Similarly, micronucleus test was conducted using ICR rats and rats were administered with 500, 1000, and 2000 mg/kg body weight ginseng adventitious root powder, the results affirmed that the frequency of micronucleated polychromatic erythrocyte cells (bone marrow of rats) did not significantly differ from the control group (Table 6). Analogous tests of a 13-week repeated dose toxicity test of ginseng adventitious root powder (300, 600, and 900 mg/kg body weight) with ICR mice did not cause death of rats. Absolute body weight, urine analysis data, hematology, blood chemistry, absolute organ weight, and histopathological findings revealed that there were no differences between the control and the treated rats, confirming the biosafety and non-toxicity of ginseng adventitious roots at an average dietary consumption level (Murthy et al. 2014e; Sivakumar et al. 2006).

Table 2 Ginsenoside contents of tissue-cultured mountain ginseng adventitious roots in comparison with cultivated ginseng

Ginsenoside	Content (mg/g dry weight)	
	Ginseng adventitious roots	Korean ginseng
Rb1	4.1	0.05
Rb2	2.3	0.06
Rb3	0.9	–
Rc	2.0	0.03
Rd	4.2	0.06
Re	0.2	0.24
Rg1	0.19	0.17
Rg2	0.38	–
Rg3	11.2	–
Rh1	0.49	–
Rh2	3.8	–
Rf	1.64	0.03

Analysis was carried out by the Korea Food Research Institute, Sungnam-si, Republic of Korea

Table 3 Chemical constituents of ginseng adventitious roots

Parameters	Units
pH	5.62 pH units
Calories	353 Cal/100 g
Calories of fat	5.006 Cal/100 g
Fat	0.54 g/100 g
Saturated fatty acid	56.7 g/100 g fat
Cholesterol	0.54 mg/100 g
Carbohydrates	58.8 g/100 g
Total dietary fiber	28.1 g/100 g
Total sugars	3.61 g/100 g
Moisture	1.19 g/100 g
Total ash	11.7 g/100 g
Protein	27.8 g/100 g
Vitamin A	20.0 IU/100 g
Vitamin C	39.6 mg/100 g
Sodium	107 mg/100 g
Calcium	464 mg/100 g
Iron	11.9 mg/100 g

The above analysis is carried out by US FDA

Table 4 In vitro reverse mutation tests on *Salmonella typhimurium* and *Escherichia coli* treated with ginseng adventitious roots without S-9 mix and with S-9 mix

Without S-9 mix					
Dose (μg/plate)	Number of revertants/plate (mean ± SD $n = 3$)				
	Base pair substitution type			Frame shift type	
	TA100	TA1535	WP2*uvr*A$^-$	TA98	TA 1357
0	116 ± 6	9 ± 3	34 ± 4	45 ± 2	8 ± 1
312.5	117 ± 17	10 ± 1	28 ± 3	51 ± 7	6 ± 2
625	120 ± 8	10 ± 2	29 ± 5	49 ± 8	7 ± 1
1250	107 ± 12	12 ± 2	30 ± 9	50 ± 3	8 ± 2
2500	123 ± 10	10 ± 1	24 ± 3	48 ± 3	8 ± 1
5000	106 ± 12	11 ± 2	27 ± 5	42 ± 4	7 ± 1
Positive Cont.	489 ± 25	242 ± 10	265 ± 5	417 ± 15	286 ± 14
Strain		Positive control		Concentration	
TA100		2-aminoanthracane (2-AA)		0.01 μg/plate	
TA 1535		2-aminoanthracane (2-AA)		1.0 μg/plate	
WP2*uvr*A$^-$		2-aminoanthracane (2-AA)		0.01 μg/plate	
TA 98		2-aminoanthracane (2-AA)		0.1 μg/plate	
TA 1537		2-aminoanthracane (2-AA)		80 μg/plate	
With S-9 mix					
Dose (μg/plate)	Number of revertants/plate (mean ± S. D. $n = 3$)				
	Base pair substitution type			Frame shift type	
	TA100	TA1535	WP2*uvr*A$^-$	TA98	TA 1357
0	116 ± 6	13 ± 2	34 ± 3	45 ± 2	8 ± 1
312.5	109 ± 10	10 ± 1	36 ± 4	51 ± 7	6 ± 2
625	111 ± 9	13 ± 2	25 ± 6	45 ± 3	9 ± 3
1250	129 ± 12	9 ± 2	31 ± 0	49 ± 5	6 ± 1
2500	104 ± 6	9 ± 2	28 ± 6	44 ± 4	7 ± 0
5000	124 ± 6	12 ± 1	25 ± 5	50 ± 3	9 ± 1
Positive Cont.	484 ± 26	246 ± 6	286 ± 11	436 ± 7	282 ± 15
Strain		Positive control		Concentration	
TA100		2-aminoflurene (AF-2)		1.0 μg/plate	
TA 1535		2-aminoflurene (AF-2)		1.0 μg/plate	
WP2*uvr*A$^-$		2-aminoflurene (AF-2)		10 μg/plate	
TA 98		2-aminoflurene (AF-2)		0.5 μg/plate	
TA 1537		2-aminoanthracane (2-AA)		2.0 μg/plate	

Efficacy profile of ginseng adventitious root biomass produced in vitro

Panax ginseng has a long history of medicinal use and one of the most popular and best selling medicine and health food worldwide (Ernst 2010; Lee and Son 2011). Many clinical studies on ginseng have been performed to characterize its therapeutic properties which include diabetes, hypertension, physical performance, sexual function, and treating cancer (Steve Helms 2004; Jin et al. 2016; Jang et al. 2008; Kim et al. 2011; Lee and Son 2011; Shergis et al. 2013). In the similar lines, various in vitro, animal studies and randomized double-blinded, placebo clinical studies in some cases have been conducted to prove the efficacy and safety of tissue-cultured ginseng adventitious roots and antioxidant, antidiabetic, antifibrotic, anti-inflammatory, anticancer, and hepatoprotective activities (Table 7).

Antioxidant and antifibrotic activities of ginseng adventitious roots

Lim et al. (2007) demonstrated the anti-oxidant activities of hot water extract of ginseng adventitious roots; they assayed 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging activity and were compared with that of N-acetyl cysteine (standard). The radical scavenging of ginseng adventitious roots was similar to 1 mM N-acetyl cysteine, which was equivalent to 0.15 mg/ml. Lim et al. (2007) also displayed reactive oxygen species scavenging activity of ginseng adventitious root extract by dichlorofluorescence assay using human heptoma HepG2 cell cultures.

Lim et al. (2007) demonstrated the anti-fibrotic effects of hot water extract of ginseng adventitious roots and investigated the possible mechanisms on carbon tetrachloride (CCl4)-

Table 5 Chromosome aberration tests on Chinese Hamster Lung (CHL) cultured cells treated with ginseng adventitious roots

S-9 mix	Test item	Dose (μg/plate)	Chromosome aberration/100 metaphase cells (mean ± SD)
S-9 mix (−) 6 + 18 h	CMC	0	1.5 ± 0.7
	TCMGARs	150	1.5 ± 0.0
		300	1.0 ± 0.4
		600	0.5 ± 0.2
	MMC	0.05	26.5 ± 2.1
S-9 mix (+) 6 + 18 h	CMC	0	1.0 ± 0.0
	TCMGARs	150	1.5 ± 0.7
		300	2.0 ± 0.0
		600	1.0 ± 0.0
	B[a]P	20	28.5 ± 0.7
S-9 mix (−) 24 + 0 h	CMC	0	0.5 ± 0.2
	TCMGARs	150	1.0 ± 0.0
		300	1.5 ± 0.7
		600	1.0 ± 0.0
	MMC	0.05	36.0 ± 2.8
S-9 mix (−) 24 + 0 h	CMC	0	0.0 ± 0.0
	TCMGARs	150	1.0 ± 0.0
		300	1.0 ± 0.4
		600	0.5 ± 0.2
	MMC	0.05	27.2 ± 2.1

CMC carboxymethylcellulose sodium salt, *MMC* mitomycin C, *B(a)P* benzo(a)pyrene

induced hepatotoxicity mice model. Serum aspertate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) activity levels were lowered by 25, 21, 11% for silymarin (drug)-treated group and by 48, 39, 14% for ginseng adventitious root extract-treated group compared to carrier treated alone. Lim et al. (2007) also demonstrated pro-fibrotic gene (TGF-β1, TIMP-1, and alpha smooth muscle actin) expression increased following the CCl_4 treatment, but both silymarin and ginseng adventitious root extract treatments decreased the expression of these genes by 20 to 50%. These experiments clearly demonstrated the antifibrotic activity ginseng adventitious roots.

Antidiabetic activity of ginseng adventitious roots

Efficacy of ginseng adventitious root extract on hyperglycemia in streptozotocin (STZ)-induced Sprague-Dawley diabetic rats was revealed by Murthy et al. (2014c). They observed consistent increase in body weight over the time period with control group of normal rats, whereas significant decrease in body weight in STZ-induced group of rats. The body weight of the ginseng adventitious root extract-administered groups was decreased when compared to the normal group; however, they observed considerable increase in body weight of the groups of rats administered with 250 and 500 mg/kg of ginseng adventitious root extract. The results demonstrated the significant restoration in body weight of rats administered with ginseng adventitious root extract. Similarly, Murthy et al. (2014c) assessed the changes in blood glucose levels in diabetic rats which were administered with ginseng adventitious root extract. Blood glucose levels increased significantly in the STZ-control groups of rats compared to the control group of rats, whereas the groups of rats administered with ginseng adventitious root extract depicted reduction in blood glucose levels after 1 week of sample administration when compared to STZ-control groups.

Anti-platelet and peripheral blood circulation activities of ginseng adventitious roots

Jeon et al. (2008) investigated the effects of 70% ethanol extracts of ginseng adventitious roots along with Korean red ginseng and Korean white ginseng on agonist-induced platelet aggregation and activation in human whole blood. The IC_{50} values for ginseng adventitious roots along with Korean red ginseng and Korean white ginseng were 1.159, 3.695, and 4.978 mg/ml for collagen-induced aggregation, 0.820, 2.030, and 4.743 mg/ml for arachidonic acid-induced aggregation,

Table 6 Micronucleus tests on male ICR mice treated with ginseng adventitious root powder

Test items	Groups	Dose (mg/kg)	Animal number	Sampling time (h)	PCE/(PCE + NCE) (mean ± SD)	MNPCE/1000PCE (mean ± SD)
Saline	G1	0	6	48	0.483 ± 0.021	0.83 ± 0.41
Ginseng adventitious root powder	G2	500	6	48	0.492 ± 0.016	0.67 ± 0.26
	G3	1000	6	48	0.485 ± 0.019	0.75 ± 0.42
	G4	2000	6	48	0.495 ± 0.014	0.92 ± 0.38
MMC	G5	2	6	24	0.493 ± 0.006	84.50 ± 6.86

MNPCE micronucleated polychromatic erythrocyte, *PCE* polychromatic erythrocyte, *NCE* nonchromatic erythrocyte, *MMC* mitomycin C

Significance $P < 0.01$ by Chi-square test

Table 7 Efficacy tests of ginseng adventitious roots obtained from in vitro culture

Sl. No.	Types of efficacy tests	References
1	Anti-oxidant activity	Ali et al. (2006); Lim et al. (2007)
2	Anti-diabetic activity	Murthy et al. (2014c)
3	Anti-fibrotic activity	Lim et al. (2007)
4	Antihypertensive and vasodilatory effects	Hong et al. (2008)
5	Anti-inflammatory activity	Yu et al. (2015)
6	Augmentation of peripheral blood flow	Lee et al. (2011)
7	Hepatoprotective activity	Murthy et al. (2014d)
8	Inhibition of cancer cell proliferation	Oh et al. (2006)
9	Inhibition of platelet aggregation activity and activation in human whole blood	Jeon et al. (2008); Lee et al. (2011)
10	Stimulation of immune cells	Oh et al. (2006)
11	Inhibition of L-DOPA oxides activity (skin whitening activity)	Xu et al. (2008)
12	Treatment of erectile dysfunction	Kim et al. (2009)
13	Treatment of hyperlipidemia	Lee et al. (2003)
14	Treatment of spermatogenic disorders	Park et al. (2006)

and 1.070, 2.617, and 2.954 mg/ml for adenosine-5′-diphosphate-induced aggregation, respectively.

Lee et al. (2011) investigated the effects of ethyl acetate extract of ginseng adventitious roots in in vitro antiplatelet activity in whole human blood and its effect on peripheral blood flow in mice. They reported that ethyl acetate extracts of ginseng adventitious roots inhibited platelet aggregation with IC_{50} values 271, 180, 147 μg/ml induced by collagen, adenosine-5′-diphosphate, and arachidonic acid, respectively. Furthermore, ethyl acetate extracts of ginseng adventitious roots improved the peripheral circulatory disturbances by improving vascular blood flow.

Anti-inflammatory activity of ginseng adventitious roots

Yu et al. (2015) investigated anti-inflammatory potential of ginsenosides derived from ginseng adventitious root extract in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. They detected an elevated production of nitric oxide (NO) in the RAW 264.7 cells in response to stimulation with LPS, through Griess reagent assay (NO detection assay). However, pretreatment of RAW 264.7 cells with ginseng adventitious root extract inhibited the production of NO through the suppression of inducible NO synthase gene expression. Furthermore, they have demonstrated the LPS-induced gene expression and reduction in production of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) by the treatment with ginseng adventitious root extract.

Hepatoprotective activity of ginseng adventitious roots

Murthy et al. (2014d) demonstrated the hapatoprotective activity of ginsenosides obtained from *Panax ginseng* adventitious roots against carbon tetrachloride (CCl4)-treated hepatic injury in rat model. They reported administration of ginseng adventitious root extract as well as cultivated ginseng extract significantly prevented CCl_4-induced elevation of AST, ALT, and ALP indicating the hepatoprotective activity of ginseng adventitious roots and also the cultivated ginseng. The administration of 300 mg/kg ginseng adventitious roots and 100 mg/kg cultivated ginseng root extract significantly lowered the AST, ALT, and ALP.

Efficacy of ginseng adventitious roots in improving sexual dysfunctions

Park et al. (2006) examined the possibility of using ginseng adventitious roots as fertility agent. They administered ginseng adventitious root powder to 7-week-old 'Sprague-Dawley' male rats orally over a 6-week period and reported the number of sperms in the testes and epididymis was significantly higher than the control. They also carried out a histological examination and did not observe any morphological changes in the testes from the ginseng adventitious root powder-treated rats.

Kim et al. (2009) investigated the effects of ginseng adventitious root extract on male patients with erectile dysfunction. They conducted a double-blind, placebo-controlled study in patients experiencing erectile dysfunction over the course of 8 weeks. The effects of ginseng adventitious root extract and the placebo were analyzed using the Korean version of the International Index of Erective Function (IIEF) questionnaire. The reported erectile function and overall satisfaction scores after medication were significantly higher in the ginseng adventitious root extract treatment group than the placebo group

($P < 0.05$). These investigations suggest the efficacy of ginseng adventitious roots in improving sexual functions.

Stimulation of immune cells and inhibition of cancer cell proliferation activities of ginseng adventitious roots

Oh et al. (2006) evaluated the ability of water extracts of ginseng adventitious roots in stimulation of immune cells. They reported that lymphocyte subpopulation in mouse splenocytes in vivo was significantly increased by the administration of ginseng adventitious root extract (27.4 mg/kg body weight of mouse). Interleukin-2 and γ-interferon in the mice serum were increased up to 30% in ginseng adventitious root extract-treated mice. Oh et al. (2006) also reported the inhibition of cancer cell proliferation with the treatment of ginseng adventitious root extracts. They demonstrated that ginseng adventitious root extract significantly retarded the cell proliferation of human acute promyelocytic (HL60), human histiocytic (Up37), and mouse lymphoctytic (L1210) leukemia cell lines in vitro at concentrations over 2.74–13.7 mg/ml. In addition, they showed increased expression of the p53 gene and protein in cultured U937 leukemia cell lines by treating with ginseng adventitious root extracts (1.37 mg and 2.74 mg/ml).

Conclusions

Ginseng (*Panax ginseng*) is a vital medicinal plant which has been a chief part of theraupetics since time immemorial in Chinese and Oriental system of medicine as it possesses very important pharmacological ingredients such as ginsenosides. Ginseng is a key component in the preparation of various pharmaceuticals, cosmetics, and nutraceuticals or functional foods in recent days. Wild mountain ginseng which exists in Korea, China, and Japan is considered as rare, precious commodity, and it has been subjected for tissue culture processes for the production of ginseng biomass using large-scale bioreactors (Paek et al. 2009; Murthy et al. 2014a). For the use of tissue culture-derived ginseng biomass (cell, hairy root, and adventitious roots) by pharmaceutical and food industry, rigorous evaluation for their quality, safety, and efficacy is necessitated (Murthy et al. 2015, 2016). The quality and toxicological assessment of ginseng adventitious roots have been carried out by Korean and American Food and Drug Administration, and based on such evaluation, Korean Food and Drug Administration (KFDA) and United States Food and Drug Administration (USFDA) have given approval for the commercial production of ginseng adventitious roots and their products (Approval No. 2030950, dt. 06/07/2002). Research investigations have been carried out by Korean researchers on the efficacy of ginseng adventitious roots since 2002 including in vitro, animal and randomized double-blinded, placebo clinical studies and they have demonstrated usefulness of the plant cell and organ culture technology for the production of ginseng raw material and its products.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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