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Biofunctional characterization of red, black and white ginseng (*Panax ginseng* Meyer) root extracts

Caracterización biofuncional de extractos de raíz de ginseng rojo, blanco y negro (*Panax ginseng* Meyer)

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Abstract

Panax ginseng is used in traditional medicinal systems since ancient times. Countless health benefits are contributed by numerous phytoconstituents. In market, three preparations of ginseng that is white, red and black are available for consumers. Current study was aimed to contrast and compare these three types of ginseng. An organized characterization of the biofunctional activities (antioxidant, antiglycation, enzymes inhibitory, antiamnestic, cytotoxic, thrombolytic, antibiofilm) of red, black, and white ginseng root extracts and fractions was performed. Antioxidant activity, phenolic and flavonoid contents were assessed by using free radical scavenging, Folin-Ciocalteu reagent and aluminum chloride colorimetric assays. Antiglycation and enzyme (alpha amylase, alpha glucosidase and acetylcholinesterse) inhibition activities were tested by prescribed methods along with cytotoxic (antihemolytic profile), thrombolytic activity, and biofilm growth inhibition assays. Red and black ginseng indicated maximum phenolic contents (37.26 ± 1.563 g GAE) and flavonoid contents (149.4 ± 2.032 g CE) respectively. The DPPH scavenging ability (62.84%) of red ginseng was higher than black and white ginseng. Significant antidiabetic activities were exhibited by black and white ginseng. The highest antihemolytic (71.2%) and thrombolytic (87%) actions were shown by black and red ginseng respectively. Red and white ginseng maximally inhibited the growth of *Pasteurella multocida* while in the case of *Staphylococcus aureus*, red ginseng showed optimum antibiofilm activity. The present study demonstrates that all three preparations of *P. ginseng* have effective yet variable functional characteristics that warrant further exploration.

Keywords: *P. ginseng*, antioxidant, enzyme inhibition, antidiabetic, phenols, biofilm.

Resumen

Panax ginseng se usa en los sistemas medicinales tradicionales desde la antigüedad. Numerosos beneficios para la salud son aportados por numerosos fitoconstituyentes. En el mercado, tres preparaciones de ginseng que son blancas, rojas y negras están disponibles para los consumidores. El estudio actual tenía como objetivo contrastar y comparar estos tres tipos de ginseng. Se realizó una caracterización organizada de las actividades biofuncionales (antioxidante, antiglicación, enzimas inhibidoras, antiamnésicas, citotóxicas, trombolíticas, antibiofilm) de extractos y fracciones de raíz de ginseng rojo, negro y blanco. Actividad antioxidante, fenólico y flavonoide los contenidos se evaluaron mediante la eliminación de radicales libres, el reactivo Folin-Ciocalteu y los ensayos colorimétricos de cloruro de aluminio. Las actividades de antiglicación y de inhibición de enzimas (alfa amilasa, alfa glucosidasa y acetilcolinesterasa) se probaron mediante métodos prescritos junto con citotóxico (perfil antihemolítico), actividad trombolítica y ensayos de inhibición del crecimiento de biopelículas. Rojo y azul. ack ginseng indicó contenidos máximos fenólicos (37.26 ± 1.563 g GAE) y contenido de flavonoides (149.4 ± 2.032 g CE) respectivamente. La capacidad de eliminación de DPPH (62.84%) del ginseng rojo fue mayor que el ginseng blanco y negro. El ginseng blanco y negro exhibió actividades antidiabéticas significativas. Las acciones antihemolíticas (71,2%) y trombolíticas (87%) más altas se mostraron con ginseng negro y rojo respectivamente. El ginseng rojo y blanco inhibió al máximo el crecimiento de *Pasteurella multocida*, mientras que en el caso de *Staphylococcus aureus*, el ginseng rojo mostró una actividad óptima de antibiofilm. El presente estudio demuestra que las tres preparaciones de *P. ginseng* tienen características funcionales eficaces pero variables que justifican una mayor exploración.

Palabras clave: *P. ginseng*, antioxidante, inhibición enzimática, antidiabético, fenoles, biopelícula.

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1 Introduction

Panax ginseng (ginseng), a member of family Araliaceae is used in traditional Chinese, Japanese, and Korean medicinal systems since ancient times. Ginseng is the most familiar medicinal plant due to its countless health benefits. Ginseng comprises 8-14% crude protein, 60% carbohydrates, 1-3% lipids, 4-6% ash, 3-8% crude saponin including other chemicals like phenolic and flavonoid contents. Commonly, ginseng roots are well known because of its chemically active ginsenosides. More attention has been occupied by phenolic compounds due to the biochemical and therapeutic assets like antioxidant, antidiabetic and anticancer as well as their capability to minimize hypertension (Chung *et al*., 2015; Jin *et al*., 2019). It strengthens spleen and lungs, stimulates biological secretion, and has a soothing effect on cardiovascular and nervous systems (Zhao *et al*., 2019). Ginsenosides, the chief phytoconstituent, are steroid saponins. In vitro studies showed that ginsenosides Rg3 and Rh-2 are used for cancer therapy in China (Chen *et al*., 2015; Shahrajabian *et al*., 2019a).

P. ginseng is an important antioxidant and acts as an immunomodulator to sustain healthy and long lifespan (Ahn *et al*., 2013) as it contains flavonoids (Jung *et al*., 2005), and phenol like maltol (Lee *et al*., 2015) which are very effective free radical scavengers. Saponins inhibit abnormal hepatic enzyme levels caused by a cholesterol-rich diet. Specific attributes of ginseng include diabetes repressive, antihyperlipidemic, and immune enhancer roles (Uluisik and Keskin, 2016; Shahrajabian *et al*., 2019b) along with improvement of stress, restrictions of DNA disintegration, kidney damage and allergic disorders (Han and Kim, 2020). Ginseng prevents reactive oxygen species (ROS) generation and apoptosis (Shin *et al*., 2014). Three preparations of ginseng are available for consumers. White ginseng is prepared from fresh ginseng by drying in the sunshine, steaming of fresh type produces red ginseng, and black ginseng is developed from white type by steaming process (Jin *et al*., 2015). Although different parts of *P. ginseng* have been extensively studied for diverse biological activities, however, there is no comparative analysis available to date that focused on several types of ginseng. An organized characterization of the biofunctional activities (antioxidant, antiglycation, enzymes inhibitory, antiamnestic, cytotoxic, thrombolytic, antibiofilm) of red, black, and white ginseng root extracts and fractions was performed.

2 Materials and methods

2.1 Sample collection and processing

The four-year sample of red, black, and white *P. ginseng* preparation was collected from the local food market, Lahore, Pakistan. The sample was cleaned, and grind to fine powder after identification and authentication at the Department of Botany, University of Agriculture, Faisalabad. The powdered samples were extracted in methanol (1:6 ratio; 1 g plant material: 6 mL methanol) by incubating the samples in MeOH for 3 days. Residues after filtration, were again extracted in MeOH for three days at room temperature. The same procedure was repeated. Viscous extracts (filtrate) obtained after thrice methanolic extractions were dried on a water bath. This procedure was performed thrice after four days and extracts were stored at -4 ºC. The MeOH extracts were mixed with distilled water. Fractions were prepared in solvents (*n*-hexane, *n*-butanol, ethanol, methanol, ethyl acetate, and chloroform). The final yield calculated as following: *n*-hexane (25 g), *n*-butanol (20 g), ethanol (60 g), ethyl acetate (5 g), chloroform (10 g) and aqueous (10 g) (Mehmood *et al*., 2012).

2.2 Anti-oxidant contents and activity

2.2.1 Phenolic Contents (PC)

Phenolic contents were assessed as described by Chaharedhi *et al*. (2009). Briefly, root extracts and fractions were combined with reagent, then sodium carbonate (20%) was added. Mixture was heated at 37 ºC, cooled and absorbance at 750 nm was measured. Results were reported as g gallic acid equivalent (GAE) /100 g dry weight (DW).

2.2.2 Flavonoid Contents (FC)

FC was assessed by using aluminum chloride colorimetric assay (Siddique *et al*., 2010). Test samples, distilled water, and $NaNO_2$ were mixed and after five minutes 300 μL $AlCl_3$ and 1 mL NaOH were added and incubated at room temperature. The absorbance was checked at 510 nm

by spectrophotometer and FC was expressed as g catechin equivalents (CE) /100 g dry weight (DW).

2.2.3 DPPH radical scavenging assay

Free radical scavenging activity was measured according to the procedure described by Souri *et al*. (2008). 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was mixed with extracts. Absorbance was measured at 515nm. Percent radical scavenging concentration was determined as percent radical scavenging or IC50 = 100 x (A blank - A sample / A blank).

2.3 Anti-diabetic activity

2.3.1 Antiglycation activity

Antiglycation activity was tested by their efficacy to control the glycation of bovine serum albumin (BSA). BSA was dissolved in a solution containing glucose, phosphate buffer (pH 7.4), and test samples. The mixture was incubated at 37 ºC for 10 days. Metformin was positive control (Matsuda *et al*., 2003). Percent inhibition formula: (Absorbance blank sample - absorbance test sample/Absorbance blank sample) x 100.

2.3.2 α-amylase inhibition assay

Sodium phosphate buffer solution of pH 6.9, 0.1 U/mL α-amylase (porcine pancreatic enzyme, Sigma Aldrich), test samples, and substrate (starch) were incubated. The reaction mixture was heated following the addition of DNS (3,5-dinitrosalicylic acid). Absorbance was measured at 540 nm. Percent inhibition: (Absorbance control - Absorbance sample/Absorbance control) $\times$ 100. Synthetic inhibitor acarbose was positive control (Apostolidis *et al*., 2006).

2.3.3 Alpha-glucosidase inhibitory test

The reaction mixture containing sample (120 μL), α-glucosidase (2.01 U/mL) and phosphate buffer (1000 μL) were shifted to test tubes and for 21-25 minutes, it was kept at room temperature. p-nitrophenyl-α-D-glucopyranoside (650 μL) was dissolved in PBS after incubation time and this was kept at 28°C for 10 minutes (Apostolidis *et al*., 2006). Quantification was done at 590 nm through a spectrophotometer. Acarbose and methanol were positive control and negative control respectively. % inhibition = 1 - [A blank - A sample /A blank] $\times$ 100.

2.4 Antiamnestic activity - Acetylcholinesterase inhibition assay

Test samples were incubated with phosphate buffer (pH 8). Electrophorus electricus (electric eel) 0.0025U/mL acetylcholinesterase concentration (Sigma-Aldrich Germany), and 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) stock solution, substrate stock solution (acetylcholine iodide) was added. Absorbance was measured at 412nm. Physostigmine was positive control and as a negative control, reaction mixture without sample was used. Percentage inhibition: (Absorbance control - Absorbance sample)/Absorbance control x 100. (Rahman *et al*., 2001).

2.5 Cytotoxic potential - Anti-hemolytic activity

Blood samples were centrifuged at 1500 rpm, supernatants (plasma) were discarded and pellets were washed with phosphate buffer saline (PBS; pH 7.4) thrice. The cells were mixed with test samples and incubated. Positive control (Triton X-100) and negative control (PBS) were included. Optical densities were measured at 576 nm. Hemolysis (%): (Absorbance test sample - Absorbance negative control/Absorbance positive control - Absorbance negative control) $\times$ 100 (Powell *et al*., 2000).

2.6 Thrombolytic activity

Blood in micro-centrifuge tubes was kept at room temperature to develop a clot. The serum was removed and clot weight was determined. Extracts were added and incubated to observe clot lysis. Post-incubation, the weight of tubes was measured to check the weight variation upon clot disturbance. Positive (streptokinase) and negative (distilled water) controls were included. The calculation was done as: Clot lysis % = W (before lysis) - W (after lysis)/ W (before lysis) x 100 (Hossain *et al*., 2012).

2.7 Biofilm inhibitory assay

Bacterial strains and root extract were mixed in a nutrient medium and incubated in 96 well microtiter plates for 4-24 hours. Plate was washed with phosphate buffer saline, and crystal violet (CV) was

added. Glacial acetic acid was used to solubilize CV. Absorbance was quantified at 630 nm. Ampicillin was positive control whereas, sample without root extract was used as the negative control (Dheepa *et al*., 2011; Sabir *et al*., 2014). Percentage biofilm inhibition: (OD of control-OD of sample/ OD of control) × 100.

2.8 Statistical analysis

Each measurement was the average of three repeats and the data were represented as mean ± standard error (SE) or percentage. SPSS (Statistical Packages for Social Sciences, version 16.0, 2003 ® SPSS Inc., Chicago, IL, USA) was used for data interpretation with a level of significance at $p< 0.05$ using analysis of variance technique.

3 Results and discussion

3.1 Antioxidant activity

Results of antioxidant contents and activity are presented in table 1. The *n*-hexane fraction of red ginseng showed significantly higher PC 37.26 ± 1.56 g GAE. The ascending order of PC for other fractions of red ginseng was ethyl acetate<ethanol<chloroform<*n*-butanol<aqueous<methanol. Phenolic content of fractions of black and white ginseng was in the range of 3.27 ± 1.19 to 11.7 ± 1.56 and 1.99 ± 1.16 to 7.18 ± 1.03 g GAE respectively. The trend in ascending order for black ginseng was ethyl acetate<*n*-butanol<*n*-hexane<aqueous<ethanol<chloroform<methanol and that for white ginseng was aqueous<*n*-butanol<ethyl acetate<*n*-hexane<ethanol<chloroform<methanol.

The secondary metabolites of plant origin are dynamic molecules with diverse potentials against biological oxidation reactions. They are exogenous defense system for humans against reactive peroxide and highly reactive hydroxyl radicals (Dhawan and Jain, 2005). Contrary to current results, an earlier study reported 0.42% PC in ginseng roots (Chung *et al*., 2015). The major difference in results may be because total phenolic contents in ginseng roots are varied depending upon different extraction methods, post-extraction treatment and germination conditions (Martínez-Ruíz *et al*., 2018; Ji *et al*., 2020; Rojo-Gutiérrez *et al*., 2020). Another study by Kim (2016) described PC in various parts of ginseng in the range of 10.46 - 95.98 g GAE. Methanol and ethanol fractions of red ginseng exhibited the highest FC of 134.03 ± 2.05 g CE and 134.01 ± 2.56 g CE respectively. FC of other fractions decreased in the order aqueous>ethyl acetate>*n*-butanol>chloroform>*n*-hexane. Ethanol fraction of black ginseng contained maximum FC by 149.4± 2.03 g CE. Other fractions of black ginseng for FC decreased in the order chloroform >*n*-butanol >ethyl acetate >*n*-hexane. FC of fractions for white ginseng was in the range of 2.33 ± 1.24 to 99.7 ± 2.76 g CE and methanol fraction contained the highest contents of flavonoids. Though, antioxidant properties of ginseng are mostly attributed by phenolic compounds but flavonoids significance cannot be ignored. In a previous study, flavonoid contents in ginseng ranged between 59.74 and 137.30 g CE. Ginseng leaves had greater flavonoid contents than root extracts. Amongst the major sub-groups of flavonoids in ginseng, flavanol contents were maximum in leaves, and catechins were mainly present in ginseng roots (Kim, 2016). The range of percent DPPH scavenging ability of red ginseng fractions was from 53.12% to 62.84%, that of black ginseng was 11.75 - 40.15% and that of white ginseng was 17.56 - 45.8%.

Table 1. Phenolic contents, flavonoid contents and DPPH radical scavenging assay.

Extract/ fractions	PC			FC			%DPPH		
	RGE	BGE	WGE	RGE	BGE	WGE	RGE	BGE	WGE
Methanol	6.43 ± 1.03	11.7 ± 1.56	7.18* ± 1.03	134.03* ± 2.05	142* ± 2.57	99.7* ± 2.76	62.03*	30.93	45.8*
Ethanol	4.72 ± 1.75	6.17 ± 1.44	3.26 ± 1.17	134.01* ± 2.56	149.4* ± 2.03	71.57* ± 2.97	62.07*	40.15*	43.12
Ethyl acetate	3.19 ± 1.18	3.27 ± 1.19	2.46 ± 1.06	17.47± 2.56	8.56 ± 2.58	3.45 ± 2.35	57	11.75	31.46
***n*-hexane**	37.26* ± 1.56	4.04 ± 1.34	3.2 ± 1.18	2.01 ± 1.17	1.76 ± 2.19	81.99* ± 1.29	53.12	11.93	17.56
***n*-butanol**	5.3 ± 1.32	3.86 ± 1.12	2.41 ± 1.12	5.84± 2.76	14.3 ± 2.54	2.33 ± 1.24	62*.84	32.39	20.26
Chloroform	5.3 ± 1.13	9.19* ± 1.11	4.14 ± 1.11	2.95 ± 2.16	61.26 ± 2.36	17.16 ± 2.61	60*	33.69	39.46
Aqueous	6.32 ± 1.18	5.2 ± 1.17	1.99 ± 1.16	53.55 ± 2.67	116.11 ± 2.81	19.98 ± 2.06	60.62*	29.25	22.32
Control	-	-	-	-	-	-	92.23	93.67	88.34

Data is represented as mean ± SE or percentage. In terms of g GAE/DW for PC (phenolic contents) and g CE/100 g DW for FC (flavonoid contents) and DPPH represented as percentage. * Significant at $p < 0.05$. RGE: Red ginseng extract, BGE: Black ginseng extract, WGE: White ginseng extract

Percent activity in increasing order for fractions of red, black and white ginseng was as follow: Red ginseng: *n*-hexane<ethyl acetate<chloroform<methanol<ethanol<aqueous<*n*-butanol, black ginseng: Ethyl acetate<*n*-hexane<aqueous<methanol<*n*-butanol<chloroform <ethanol, white ginseng: *n*-hexane<*n*-butanol<aqueous <ethyl acetate<chloroform<ethanol<methanol. Results of the current study are partly following the findings of Chung *et al*. (2015), as in their research, ginseng roots exhibited 18.08 - 25.61% antioxidant activity in methanol extracts.

3.2 Antidiabetic activity

Glycation, alpha amylase and alpha glucosidase inhibitory activities are shown in fig. 1-3. Advanced glycation end product (AGE) development can be increased beyond normal levels due to some pathologic conditions such as oxidative stress in diabetic patients due to hyperglycemia. AGEs are now identified as proinflammatory mediators in gestational diabetes. Red and black ginseng showed 28.6 - 85.36% and 41 - 94.33 percentage inhibition of glycation respectively while white ginseng indicated 39.4 - 85.06% glycation restriction. Black ginseng was the most potent inhibitor of the glycosylation process. The glycation reduction sequence was as: ethyl acetate>*n*-butanol>chloroform>*n*-hexane>methanol>aqueous>ethanol (red ginseng), chloroform>*n*-butanol>aqueous>ethyl acetate>ethanol >methanol>*n*-hexane (black ginseng) and methanol>chloroform>aqueous>ethyl acetate>*n*-hexane>*n*-butanol>ethanol (white ginseng). Earlier, the effects of ginseng extract on AGEs formation were investigated (Quan *et al*., 2013) and the glycation process was inhibited by up to 24% by ginseng root extracts. Regarding alpha amylase inhibitory experiment, 15.66 - 89.06%, 57.63 - 93.6%, and 35.63 - 80.43% inhibitions were observed for red, black, and white ginseng respectively (Fig. 2). According to previously reported data, ginsenosides Rg1, Rb1, and Rg3, active components of *P. ginseng*, showed 39.7% alpha amylase inhibition consecutively (Xiao-Ping *et al*., 2010). Ginseng is used worldwide as a medicinal plant that contains triterpenoids or ginsenosides. Ginseng exerts its antidiabetic effects either by regulating insulin secretion, and glucose absorption or by influencing antioxidant and anti-inflammatory mechanisms (Chen *et al*., 2019).

In the present research, alpha glucosidase inhibition assay was carried out to investigate the antidiabetic potential of *P. ginseng* root extracts (fig. 3).

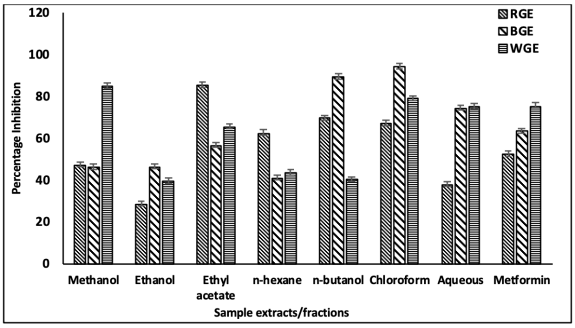

Fig. 1. Glycation inhibition. Percentage inhibition of glycation of BSA with glucose in the presence of plant extracts/fractions. Results are presented as means ± S.E (n=3). RGE: Red ginseng extract, BGE: Black ginseng extract, WGE: White ginseng extract.

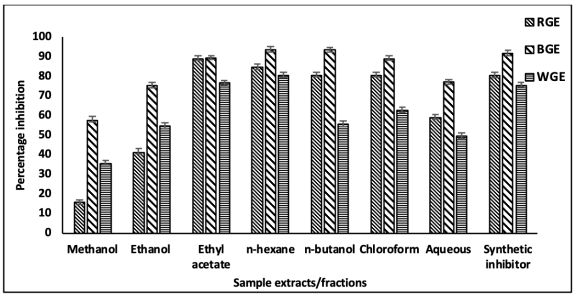

Fig. 2. Alpha amylase inhibition. Percentage inhibition of alpha amylase inhibition in the presence of plant extracts/fractions. Results are presented as means ± S.E (n=3). RGE: Red ginseng extract, BGE: Black ginseng extract, WGE: White ginseng extract.

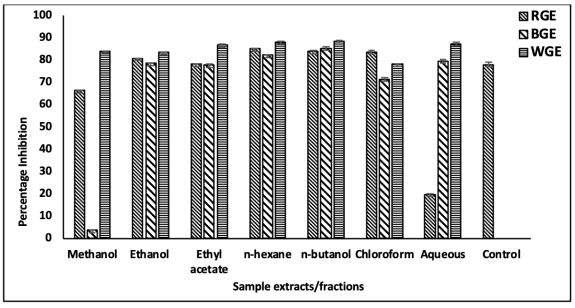

Fig. 3. Alpha glucosidase inhibition. Percentage inhibition of alpha glucosidase inhibition in the presence of plant extracts/fractions. Results are presented as means ± S.E (n=3). RGE: Red ginseng extract, BGE: Black ginseng extract, WGE: White ginseng extract.

n-butanol fractions of black and white ginseng exhibited maximum percentage inhibitions i.e. 85.66% and 88.87% respectively while optimum α-glucosidase percentage inhibition for red ginseng was shown by *n*-hexane fraction (85.26%). *n*-hexane fraction of red ginseng exhibited utmost percentage inhibition. *n*-butanol fraction has the greatest percentage inhibition for black ginseng as well as both these fractions gave maximum percentage inhibition for white ginseng. Alpha glucosidase secreted from intestinal cells is involved in hydrolyzing carbohydrates to simple sugar thus increasing glucose level leading towards diabetes. It is important to develop enzyme inhibition therapy in diabetic patients to prevent hyperglycemia. Hence, it is one of the important enzymes that play a role to maintain blood glucose levels in diabetes mellitus (Subramanian *et al*., 2008). Recently, when Park *et al*. (2020) explored the antidiabetic activity of Korean red ginseng (KGC05P0) in terms of carbohydrate digestive enzymes inhibitions, it was observed that KGC05P0 inhibited α-glucosidase and α-amylase activities.

3.3 Acetylcholinesterase inhibitory activity

Ginseng was screened for its effect on the cholinergic system in vitro (Table 2). Black ginseng had maximum antiamnestic potential with 4.70% inhibition followed by white ginseng (3.5% AChE inhibition). Ethyl acetate and ethanol fractions of red and black ginseng were least (0.56%) inhibitors of AChE activity. No AChE inhibitory action was revealed by the *n*-hexane fraction of white ginseng. Although AChE inhibition by synthetic inhibitor was impressive, all the three ginsengs did not exhibit any therapeutic potency for the treatment of neurodegenerative disorders during in vitro assay. Better results would have been observed by in vivo experimentations. Natural products affect multiple physiological domains leading to an immense positive outcome. Earlier symptoms of brain deterioration are loss of function and damage to neurons. Emerging evidence supports the valued health effects of *Panax ginseng*. The neuroprotective role of *Panax ginseng* is mostly contributed by antioxidative and immunomodulatory potentials of ginsenoside. Ginsenosides modify bio-signaling, cell respiration, and death genes (Kim *et al*., 2018; Jakaria *et al*., 2019).

Lee *et al*. (2012) determined acetylcholinesterase inhibition activities of ethanol samples of black and white ginseng and found maximum inhibition with estimated IC50 values of 5.37 mg/mL and 2.64 mg/mL respectively. Recently, *Panax ginseng* increased the concentration of antioxidant enzymes and thus prevented the central nervous system from damage caused by reoxygenation injury (Shamim and Khan, 2019). Alpha glucosidase secreted from intestinal cells is involved in hydrolyzing carbohydrates to simple sugar thus increasing glucose level leading towards diabetes. It is important to develop enzyme inhibition therapy in diabetic patients to prevent hyperglycemia. Hence, it is one of the important enzymes that play a role to maintain blood glucose levels in diabetes mellitus (Subramanian *et al*., 2008).

Table 2. Antihemolytic, thrombolytic and acetylcholinesterase inhibitory profiles.

Extract/ fractions	Percentage inhibition								
	Antihemolytic assay			Thrombolytic assay			Acetylcholinesterase inhibition		
	RGE	BGE	WGE	RGE	BGE	WGE	RGE	BGE	WGE
Methanol	13.96	6	21.76	78.7	72.2	77.6*	1.31	1.31	2.4
Ethanol	19.04	11.53	9.52	74.52	86*	75.1	1.69	0.56	0.94
Ethyl acetate	40.19	31.31	7.98	69.3	77.83	74.33	0.56	2.07	2.07
***n*-hexane**	5.19	3.749	6.66	82	75.5	76*	2.07	4.7	No activity
***n*-butanol**	82*	71.2*	24.12	83	76.5	74.16	1.31	1.31	0.94
Chloroform	61.83	40.35	60.23*	86*	74	75.33	0.79	2.07	3.5
Aqueous	4.8	3.35	3.83	87*	79	71	2.82*	0.94	2.07
Control	95.31			91.3			59.5		

Data is presented as percentage inhibition value of triplicate measurements. * Significant at $p < 0.05$. RGE: Red ginseng extract, BGE: Black ginseng extract, WGE: White ginseng extract. Triton X-100, streptokinase and physostigmine were positive controls for anti-hemolytic, thrombolytic and acetylcholinesterase activities respectively.

3.4 Cytotoxic potentials - Antihemolytic activity

As shown in table 2, *n*-butanol fractions of both red (82%) and black (71.2%) ginseng showed highly significant anti-hemolytic activity while chloroform fraction of white ginseng repressed hemolysis by 60.23%. The anti-hemolytic activity of ginseng was less than that of standard agent Triton X-100. Liu *et al*. (2002) also evaluated the anti-hemolytic activity of ginsenosides and their effective immunological nature. It was perceived that ginsenosides possess the ability to shield human erythrocytes from hemolysis. The erythrocytes are susceptible to lipoperoxidation as during the respiration process high concentration of oxygen can induce oxidative stress. The subsequent cell hemolysis is a frequently encountered pathological states that can be lessened by using antioxidants. All three ginsengs in current research proved to be dynamic protectors against red cell membrane damage. Current inferences can be justified by the presence of specific chemical species in ginseng as illustrated by Li and Liu (2008). Structure-activity-relationship between ginsenosides and their antihemolytic potentials was studied. It was concluded that certain monosaccharides and hydroxyl groups play a critical role in hemolysis inhibition. Hemolysis is mainly caused by free radical-initiated membrane lipid peroxidation which is related to many pathological conditions. Erythrocyte membrane fats are more prone to ROS induced lipid peroxidation. Oxidative stress results in membrane lysis. Secondary plant metabolites are excellent protectors of lipid peroxidation.

3.5 Thrombolytic activity

The antithrombolytic saponins in *Panax ginseng* activate plasminogen which can clear the fibrin mesh (Ali *et al*., 2013; Ramjan *et al*., 2014). Maximum thrombolytic percentage inhibition was presented by almost every fraction of all the three ginsengs. Red, black and white ginseng exhibited thrombosis inhibition of 69.3 - 87%, 72.2 - 86%, and 71 - 77.6% respectively as shown in Table 2. Aqueous and chloroform samples of red ginseng presented extremely noteworthy results and ethanol fraction of black ginseng had maximum inhibitory action. Similarly, the white ginseng methanolic sample displayed the highest inhibition. However, the antithrombotic potencies of all three ginsengs were lesser than the popular anti-thrombotic agent streptokinase. However, the use of ginseng as an anti-thrombolytic agent can be safer with fewer side effects. Jin *et al*. (2007) observed the antiplatelet and antithrombotic potentials of red ginseng extracts in vitro. Red ginseng prevented platelet aggregation with an IC50 maximum value of 335 μg/mL. Antithrombotic effect of red type ginseng in the current study may be due to its capacity to minimize platelet accumulation instead of its ability to lessen coagulation as described earlier (Kwon *et al*., 2016). In recent years, cardiovascular disorders are the most severe ailments that are increasing at an alarming rate. Thrombolytic agents such as streptokinase are used to dissolve the clot. Natural compounds have antiplatelet, anticoagulant, and antithrombotic activities.

Table 3. Biofilm inhibitory assay.

Extract/ fractions	**Pasteurella multocida**			**Staphylococcus aureus**		
	RGE	**BGE**	**WGE**	**RGE**	**BGE**	**WGE**
Methanol	90.3*	76.7	84.5	89.3	86.6*	80.0
Ethanol	85.1	78.8	79.7	90.1*	78.7	82.2
n-butanol	82.0	75.9	82.2	90.6	86.2*	83.5
n-hexane	70.5	79.0	69.3	85.6	78.4	74.9
Chloroform	No activity	No activity	90.0*	82.5	75.0	82.3
Ethyl acetate	36.3	76.0	12.1	No activity	No activity	88.4*
Aqueous	64.8	84.3*	80.1	62.5	76.6	76.4

Data presented as percentage inhibition value of triplicate measurements. * Significant at $p < 0.05$. RGE: Red ginseng extract, BGE: Black ginseng extract, WGE: White ginseng extract.

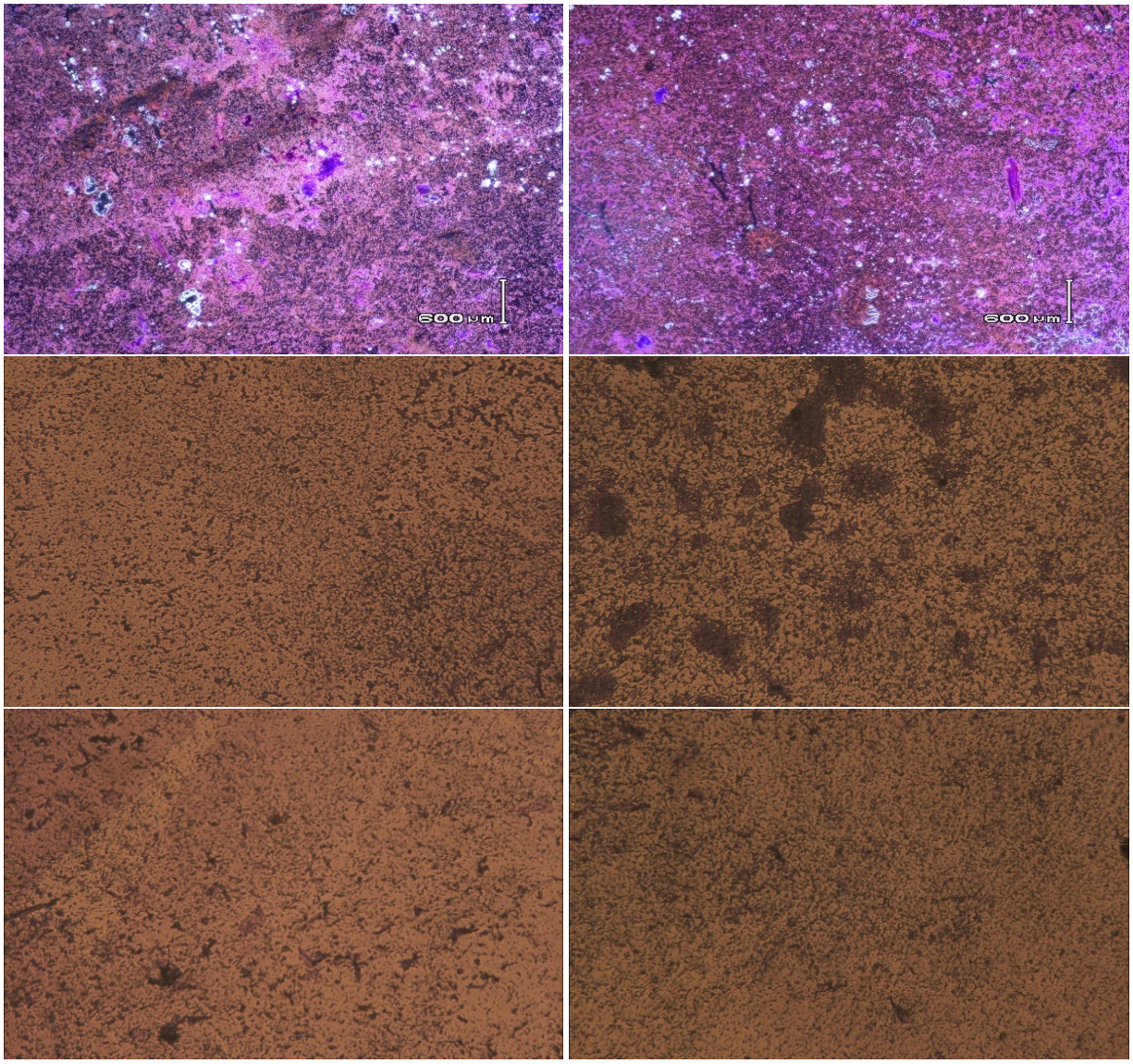

Fig. 4. (A-F) Biofilm assay - maximum growth inhibitions. A: P. multocida by methanol fraction (red ginseng), B: P. multocida by aqueous fraction (brown ginseng), C: P. multocida by chlroform fraction (white ginseng), D: S. aureus by n-butanol fraction (red ginseng), E: S. aureus by methanol fraction (black ginseng), F: S. aureus by ethyl acetate fraction (white ginseng).

3.6 Biofilm inhibitory assay

To assess the antibiofilm effect of *P. ginseng* root extracts, *Pasteurella multocida* and *Staphylococcus aureus* were used (Fig. 4 a-f). A maximum growth reduction was given by white ginseng (table 3). Red ginseng was comparatively better than black ginseng in repressing biofilm formation. Limited data is available regarding anti-bacterial potentials of three preparations of ginseng roots. Earlier Wu *et al*. (2011) investigated effects of ginseng on the formation of *P. aeruginosa* biofilms. Although ginseng aqueous extract (0.5-2.0%) did not inhibit the growth of *P. aeruginosa*, but it blocked the formation of biofilm. Aqueous ginseng (0.5%) destroyed mature biofilms. It was suggested that ginseng treatment may help to eradicate the biofilm-associated chronic infections caused by *P. aeruginosa*.

Growth reduction as exhibited in the current assay can be justified by the fact that ginseng has various

phytochemical constituents such as polyphenolic compounds, tetracyclic triterpenoid ginsenosides, and polyacetylenes due to which it showed anti-bacterial activity by suppressing defensive mechanisms of pathogens. Ginseng can repress the synthesis of protease and elastase enzyme efficacy; so, inhibited the pathogenesis mechanism of bacteria (Dhama *et al*., 2014). Recently, it was stated by Wang *et al*. (2020) that numerous phytoconstituents are responsible for antimicrobial activities of ginseng. These components in various plants inhibit microbial movement, biofilm formation and maturation, disturb lipid bilayers to create pores, efflux of cell metabolites, and ultimately destroy the cells. Another effect is by enhancing the host immune system and by weakening microbe potential to induce damage (Treviño-Garza *et al*., 2019; Ju *et al*., 2020).

Conclusions

All three preparations of *P. ginseng* exhibited promising yet inconclusive bioactivities. Significant antioxidant, antidiabetic, antihemolytic, thrombolytic, and antibacterial characteristics manifested during current research. Further explorations are warranted to bridge the conceptual and practical knowledge.

References

Ahn, H.Y., Hong, S.Y., Kim, J.Y. and Kwon, O. (2013). *Panax ginseng* extract rich in ginsenoside protopanaxatriol offers combinatorial effects in nitric oxide production via multiple signaling pathways. *Springerplus 2*, 96. https://doi:10.1186/2193-1801-2-96

Ali, M.S., Amin, M.R., Kamal, C.M.I. and Hossain, M.A. (2013). In vitro antioxidant, cytotoxic, thrombolytic activities and phytochemical evaluation of methanol extract of the *A. philippense* L. leaves. *Asian Pacific Journal of Tropical Biomedicine 3*, 464-69. https://doi:10.1016/S2221-1691(13)60097-0

Apostolidis, E., Kwon, Y.I., Shetty, K., Apostolidis, E. and Kwon, Y.I. (2006). Potential of cranberry-based herbal synergies for diabetes and hypertension management. *Asia Pacific Journal of Clinical Nutrition 15*, 433-41.

Chahardehi, A.M., Ibrahim, D. and Sulaiman, S.F. (2009). Antioxidant activity and total phenolic content of some medicinal plants in Urticaceae family. *Journal of Applied Biological Sciences 3*, 27-31.

Chen, X.P., Lin, Y.P., Hu, Y.Z., Liu, C.X., Lan, K. and Jia, W. (2015). Phytochemistry, metabolism, and metabolomics of ginseng. *Chinese Herbal Medicines 7*, 98-108. https://doi.org/10.1016/S1674-6384(15)60026-0

Chen, W., Balan, P. and Popovich, D.G. (2019). Review of ginseng anti-diabetic studies. *Molecules 24*, 4501. https://doi:10.3390/molecules24244501

Chung, I.M., Lim, J.J., Ahn, M.S., Jeong, H.N., An, T.J. and Kim, S.H. (2015). Comparative phenolic compound profiles and antioxidative activity of the fruit, leaves, and roots of Korean ginseng (*Panax ginseng* Meyer) according to cultivation years. *Journal of Ginseng Research 40*, 68-75. https://doi:10.1016/j.jgr.2015.05.006

Dhama, K., Tiwari, R., Chakraborty, S., Saminathan, M., Kumar, A., Karthik, K., Wani, M.Y., Amarpal, S.S. and Rahal, A. (2014). Evidence based antibacterial potentials of medicinal plants and herbs countering bacterial pathogens especially in the era of emerging drug resistance: An integrated update. *International Journal of Pharmacology 10*, 1-43. https://doi:10.3923/ijp.2014.1.43

Dheepa, M., Vinitha, L.R. and Appalaraju, B. (2011). Comparison of biofilm production and multiple drug resistance in clinical isolates of *Acinetobacter baumanii* from a tertiary care hospital in South India. *International Journal Pharmaceutical and Biomedical Sciences 2*, 103-7

Dhawan, V. and Jain, S. (2005). Garlic supplementation prevents oxidative DNA damage in essential hypertension. *Molecular and Cellular Biochemistry 275*, 85-94. https://doi:10.1007/s11010-005-0824-2

Han, M.J. and Kim, D.H. (2020). Effects of red and fermented ginseng and ginsenosides on allergic

disorders. *Biomolecules 10*, 634. https://doi:10.3390/biom10040634

Hossain, M.S., Chowdhury, M.E.H., Das, S. and Chowdhury, I.U. (2012). In vitro thrombolytic and anti-inflammatory activity of *Swertia chirata* ethanolic extract. *Journal of Pharmacognosy and Phytochemistry 1*, 99-104.

Iqbal, H. and Rhee, D.K. (2020). Ginseng alleviates microbial infections of the respiratory tract: a review. *Journal of Ginseng Research 44*, 194-204. https://doi:10.1016/j.jgr.2019.12.001

Jakaria, M., Kim, J., Karthivashan, G., Park, S.Y., Ganesan, P. and Choi, D.K. (2019). Emerging signals modulating potential of ginseng and its active compounds focusing on neurodegenerative diseases. *Journal of Ginseng Research 43*, 163-71. https://doi.org/10.1016/j.jgr.2018.01.001

Ji, S., Yoo, T., Jin, S., Ju, H., Eom, S., Kim, J.-S., & Hyun, T. (2020). Changes in the phenolic compounds profile, antioxidant and anti-melanogenic activity from organs of Petasites japonicas under different extraction methods. *Revista Mexicana De Ingeniería Química 19*, 1453-1464. https://doi.org/10.24275/rmiq/Bio1186

Jin, Y.R., Yu, J.Y., Lee, J.J., You, S.H., Chung, J.H., Noh, J.Y., Im, J.H., Han, X.H., Kim, T.J., Shin, K.S. and Wee, J.J. (2007). Antithrombotic and antiplatelet activities of Korean red ginseng extract. *Basic & Clinical Pharmacology & Toxicology 100*, 170-75. https://doi:10.1111/j.1742-7843.2006.00033.x

Jin, Y., Kim, Y.J., Jeon, J.N., Wang, C., Min, J.W., Noh, H.Y. and Yang, D.C. (2015). Effect of white, red and black ginseng on physicochemical properties and ginsenosides. *Plant Foods for Human Nutrition 70*, 141-45. https://doi:10.1007/s11130-015-0470-0

Jin, Y., Cui, R., Zhao, L., Fan, J. and Li, B. (2019). Mechanisms of *Panax ginseng* action as an antidepressant. *Cell Proliferation 52*, p.e12696. https://doi:10.1111/cpr.12696

Jung, C.H., Seog, H.M., Choi, I.W., Choi, H.D. and Cho, H.Y. (2005). Effects of wild ginseng (*Panax ginseng* CA Meyer) leaves on lipid peroxidation levels and antioxidant enzyme activities in streptozotocin diabetic rats. *Journal of Ethnopharmacology 98*, 245-50. https://doi:10.1016/j.jep.2004.12.030

Ju, H. J., Yoo, T., Jin, S., Kim, H. and Hyun, T. K. (2020). In vitro evaluation of the pharmacological properties of crude methanol extract and its fractions of Aconitum austrokoreense aerial parts. *Revista Mexicana De Ingeniería Química 19*, 1341-1350. https://doi.org/10.24275/rmiq/Bio1067

Kim, J.S. (2016). Investigation of phenolic, flavonoid, and vitamin contents in different parts of Korean Ginseng (*Panax ginseng* CA Meyer). *Preventive Nutrition and Food Science 21*, 263-70. https://doi:10.3746/pnf.2016.21.3.263

Kim, K.H., Lee, D., Lee, H.L., Kim, C.E., Jung, K. and Kang, K.S. (2018). Beneficial effects of *Panax ginseng* for the treatment and prevention of neurodegenerative diseases: past findings and future directions. *Journal of Ginseng Research 42*, 239-47. https://doi.org/10.1016/j.jgr.2017.03.011

Kwon, H.W., Shin, J.H., Cho, H.J., Rhee, M.H. and Park, H.J. (2016). Total saponin from Korean Red Ginseng inhibits binding of adhesive proteins to glycoprotein IIb/IIIa via phosphorylation of VASP (Ser157) and dephosphorylation of PI3K and Akt. *Journal of Ginseng Research 40*, 76-85. https://doi:10.1016/j.jgr.2015.05.004

Lee, M.R., Yun, B.S. and Sung, C.K. (2012). Comparative study of white and steamed black *Panax ginseng*, *P. quinquefolium*, and *P. notoginseng* on cholinesterase inhibitory and antioxidative activity. *Journal of Ginseng Research 36*, 93-101. https://doi:10.5142/jgr.2012.36.1.93

Lee, D., Ghafoor, K., Moon, S., Kim, S.H., Kim, S., Chun, H. and Park, J. (2015). Phenolic compounds and antioxidant properties of high hydrostatic pressure and conventionally treated ginseng (*Panax ginseng*) products. *Quality Assurance and Safety of Crops & Foods 7*, 493-500.

Li, G.X. and Liu, Z.Q. (2008). The protective effects of ginsenosides on human erythrocytes against hemin-induced hemolysis. *Food and Chemical Toxicology 46*, 886-92. https://doi:10.1016/j.fct.2007.10.020

Liu, Z.Q., Luo, X.Y., Sun, Y.X., Chen, Y.P. and Wang, Z.C. (2002). Can ginsenosides protect human erythrocytes against free-radical-induced hemolysis? *Biochimica et Biophysica Acta (BBA)-General Subjects 1572*, 58-66. https://doi:10.1016/s0304-4165(02)00281-7

Matsuda, H., Wang, T., Managi, H. and Yoshikawa, M. (2003). Structural requirements of flavonoids for inhibition of protein glycation and radical scavenging activities. *Bioorganic & Medicinal Chemistry 11*, 5317-23.https://doi.org/10.1016/j.bmc.2003.09.045

Mehmood, N., Zubair, M., Rizwan, K., Rasool, N., Shahid, M. and Ahmad, V.U. (2012). Antioxidant, antimicrobial and phytochemical analysis of *Cichorium intybus* seeds extract and various organic fractions. *Iranian Journal of Pharmaceutical Research 11*, 1145-51.

Mohsenipour, Z. and Hassanshahian, M. (2015). The effects of *Allium sativum* extracts on biofilm formation and activities of six pathogenic bacteria. *Jundishapur Journal of Microbiology 8*, e18971. https://doi:10.5812/jjm.18971v2

Park, S.J., Lee, D., Kim, D., Lee, M., In, G., Han, S.T., Kim, S.W., Lee, M.H., Kim, O.K. and Lee, J. (2020). The non-saponin fraction of Korean Red Ginseng (KGC05P0) decreases glucose uptake and transport in vitro and modulates glucose production via down-regulation of the PI3K/AKT pathway *in vivo*. *Journal of Ginseng Research 44*, 362-72. https://doi:10.1016/j.jgr.2019.12.004

Powell, W.A., Catranis, C.M. and Maynard, C.A. (2000). Design of self-processing antimicrobial peptides for plant protection. *Letters in Applied Microbiology 31*, 163-68. https://doi:10.1046/j.1365-2672.2000.00782.x

Quan, H.Y. and Do Yeon Kim and S.H.C. (2013). Korean red ginseng extract alleviates advanced glycation end product-mediated renal injury. *Journal of Ginseng Research 37*,187. https://doi:10.5142/jgr.2013.37.187

Rahman, A.U., Choudhary, M.I. and Thomsen, W.J. (2001). *Bioassay Techniques for Drug Development*. CRC Press.

Ramjan, A., Hossain, M., Runa, J.F., Md, H. and Mahmodul, I. (2014). Evaluation of thrombolytic potential of three medicinal plants available in Bangladesh, as a potent source of thrombolytic compounds. *Avicenna Journal of Phytomedicine 4*, 430-36.

Rojo-Gutiérrez, E., Buenrostro-Figueroa, J., Natividad-Rangel, R., Romero-Romero, R., Sepulveda, D., & Baeza-Jimenez, R. (2020). Effect of different extraction methods on cottonseed oil yield. *Revista Mexicana De Ingeniería Química 19*, 385-394. https://doi.org/10.24275/rmiq/Alim1704

Sabir, S., Anjum, A.A., Ijaz, T. and Ali, M.A. (2014). Isolation and antibiotic susceptibility of *E. coli* from urinary tract infections in a tertiary care hospital. *Pakistan Journal of Medical Sciences 30*, 389-95.

Shahrajabian, M.H., Sun, W. and Cheng, Q. (2019a). A review of ginseng species in different regions as a multipurpose herb in traditional Chinese medicine, modern herbology and pharmacological science. *Journal of Medicinal Plants Research 13*, 213-26. https://doi:10.5897/JMPR2019.6731

Shahrajabian, M.H., Sun, W., Cheng, Q. (2019b). The power of natural Chinese medicine, ginger and ginseng root in an organic life. *Middle-East Journal of Scientific Research 27*, 64-71. https://doi:10.5829/idosi.mejsr.2019.64.71

Shin, H.S., Yu, M., Kim, M., Choi, H.S. and Kang, D.H. (2014). Renoprotective effect of red ginseng in gentamicin-induced acute kidney injury. *Laboratory Investigation 94*, 1147-60. https://doi:10.1038/labinvest.2014.101

Shamim, M. and Khan, N.I. (2019). Neuroprotective effect of *Panax ginseng* extract against cerebral ischemia-reperfusion-injury-induced oxidative stress in middle cerebral artery occlusion models. *Facets 4*, 52-68. https://doi.org/10.1139/facets-2018-0025

Siddique, N.A., Mujeeb, M., Najmi, A.K. and Akram, M. (2010). Evaluation of antioxidant activity, quantitative estimation of phenols and flavonoids in different parts of *Aegle marmelos. African Journal of Plant Sciences 4*, 1-5.

Souri, E.G., Amin, G., Farsan, H., Jalalizadeeh, H. and Barezi, S. (2008). Screening of thirteen medicinal plants extracts for antioxidant activity. *Iranian Journal of Pharmaceutical Research 7*, 149-54.

Subramanian, R., Asmawi, M.Z. and Sadikun, A. (2008). In vitro alpha-glucosidase and alpha-amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide. *Acta Biochimica Polinica 55*, 391-98.

Treviño-Garza, M., Yañez-Echeverría, S., García, S., Mora-Zúñiga, A. and Arévalo-Niño, K. (2019). Physico-mechanical, barrier and antimicrobial properties of linseed mucilague films incorporated with *H. virginiana* extract. *Revista Mexicana De Ingeniería Química 19*, 983-996. https://doi.org/10.24275/rmiq/Bio872

Uluisik, D. and Keskin, E. (2016). Hepatoprotective effects of ginseng in rats fed cholesterol rich diet. *Acta Scientiae Veterinariae 44*, 1346.

Wang, L., Huang, Y., Yin, G., Wang, J., Wang, P., Chen, Z., Wang, T. and Ren, G. (2020). Antimicrobial activities of Asian ginseng, American ginseng, and notoginseng. *Phytotherapy Research 34*, 1226-1236. https://doi:10.1002/ptr.6605

Wu, H., Lee, B., Yang, L., Wang, H., Givskov, M., Molin, A., Hoiby, N. and Song, Z. (2011). Effects of ginseng on *Pseudomonas aeruginosa* motility and biofilm formation. *FEMS Immunology and Medical Microbiology 62*, 49-56. https://doi:10.1111/j.1574-695X.2011.00787.x

Xiao-Ping, Y.E., Chun-Qing, S.O.N.G., Ping, Y.U.A.N. and Ren-Gang, M.A.O. (2010). α-glucosidase and α-amylase inhibitory activity of common constituents from traditional Chinese medicine used for diabetes mellitus. *Chinese Journal of Natural Medicines 8*, 349-52. https://doi.org/10.1016/S1875-5364(10)60041-6

Zhao, B., Lv, C. and Lu, J. (2019). Natural occurring polysaccharides from *Panax ginseng* CA Meyer: A review of isolation, structures, and bioactivities. *International Journal of Biological Macromolecules 133*, 324-36. https://doi:10.1016/j.ijbiomac.2019.03.229