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, antiinmestatic, 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 acetylcholinesterase) 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 antihemolysis (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, antiinmestaticas, citotóxicas, trombolíticas, antibiofilm) de extractos y fracciones de raíz de ginseng rojo, negro y blanco. Actividad antioxidante, fenólico y flavonoides 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), trombólisis, actividad y biofilme crecimiento. 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.
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 NaNO2 were mixed and after five minutes 300 µL AlCl3 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 515 nm. 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 °C 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) x 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] × 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) × 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
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%.

### 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.

<table>
<thead>
<tr>
<th>Extract/fractions</th>
<th>PC</th>
<th>FC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>6.43 ± 1.03</td>
<td>134.03 ± 2.05</td>
</tr>
<tr>
<td>Ethanol</td>
<td>4.72 ± 1.75</td>
<td>134.01 ± 2.56</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>3.19 ± 1.18</td>
<td>17.47 ± 2.56</td>
</tr>
<tr>
<td><em>n</em>-hexane</td>
<td>37.26 ± 1.56</td>
<td>2.01 ± 1.17</td>
</tr>
<tr>
<td><em>n</em>-butanol</td>
<td>5.3 ± 1.32</td>
<td>5.84 ± 2.76</td>
</tr>
<tr>
<td>Chloroform</td>
<td>5.3 ± 1.13</td>
<td>2.95 ± 2.16</td>
</tr>
<tr>
<td>Aqueous</td>
<td>6.32 ± 1.18</td>
<td>53.55 ± 2.67</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Data is presented 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).

![Glycation inhibition](image1.png)

**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.

![Alpha amylase inhibition](image2.png)

**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.

![Alpha glucosidase inhibition](image3.png)

**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.
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.

<table>
<thead>
<tr>
<th>Extract/ fractions</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RGE</td>
</tr>
<tr>
<td>Methanol</td>
<td>13.96</td>
</tr>
<tr>
<td>Ethanol</td>
<td>19.04</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>40.19</td>
</tr>
<tr>
<td>n-hexane</td>
<td>5.19</td>
</tr>
<tr>
<td>n-butanol</td>
<td>82*</td>
</tr>
<tr>
<td>Chloroform</td>
<td>61.83</td>
</tr>
<tr>
<td>Aqueous</td>
<td>4.8</td>
</tr>
<tr>
<td>Control</td>
<td>95.31</td>
</tr>
</tbody>
</table>

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 antithrombotic 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>
<thead>
<tr>
<th>Extract/fractions</th>
<th>Pasteurella multocida</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RGE</td>
<td>BGE</td>
</tr>
<tr>
<td>Methanol</td>
<td>90.3*</td>
<td>76.7</td>
</tr>
<tr>
<td>Ethanol</td>
<td>85.1</td>
<td>78.8</td>
</tr>
<tr>
<td>n-butanol</td>
<td>82.0</td>
<td>75.9</td>
</tr>
<tr>
<td>n-hexane</td>
<td>70.5</td>
<td>79.0</td>
</tr>
<tr>
<td>Chloroform</td>
<td>No activity</td>
<td>No activity</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>36.3</td>
<td>76.0</td>
</tr>
<tr>
<td>Aqueous</td>
<td>64.8</td>
<td>84.3*</td>
</tr>
</tbody>
</table>

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.
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


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


