https://doi.org/10.46389/rjd-2020-1003
www.rjdnmd.org

Rom J Diabetes Nutr Metab Dis
2020; volume 27, issue 1, pages 9-15

Original Article

The Effect of Protein Fractions of Avocado (*Persea americana*) on Biochemical Parameters in a Diabetic Rat Model

Zena Aljawadi

Department of Chemistry, College of Sciences, University of Mosul, Mosul, Iraq

Correspondence to: Zena Aljawadi, Department of Chemistry, College of Sciences, University of Mosul, Mosul, Iraq. Phone: +964-770176-6612; e-mail: zena_aljawadi@yahoo.com

Received: 21 November 2019 / Accepted: 20 February 2020

Abstract

Introduction: The separation and inspection of the active protein constituents from the aqueous extract of avocado (*Persea americana*) by employing distinct biochemical ways is the primary target of this investigation. **Material and Methods:** Two compounds (A and B) were precipitated by ammonium sulfate and separated through gel filtration chromatography. **Results:** The glucose level decreased, compared to the oral administration because of the intraperitoneal administration of the protein fraction (B) and the concentrated aqueous extract. The molecular mass of the separated active protein fraction (peak B), was determined as 24,000 Daltons. Besides, certain blood components of alloxan-induced diabetic rats and normal rats were measured under the impact of the protein fraction B and the aqueous extract in doses of 50, 75, 100 and 125 mg per kilogram of body mass. The protein compound and the crude aqueous extract of the avocado with a dosage of 75 mg per kilogram of body weight significantly diminished the fasting blood sugar level in the normal rats compared to the normal set. Additionally, at the same dosage, a significant reduction in the serum total lipid and cholesterol levels was noticed. A noteworthy decline in the serum glucose, cholesterol, and total lipid levels in the diabetic rats, was beholden using the active protein in a dose of 75 mg/kg of body weight. **Conclusion:** This study showed that avocado (*Persea americana*) has the potential to be used for diabetes therapy due to active protein components.

Keywords: Avocado; *Persea Americana*; diabetes disease.

Introduction

Nearly 150 kinds of avocado can be found in tropical and subtropical regions, including *Persea Americana* [1]. In ethnomedicine, the seed of the avocado was utilized in varied applications, including treatment for dysentery, diarrhea, intestinal parasites, toothache, as well as skincare and in the beauty manufacture. Several health advantages, including weight loss and controlling human weight, have been exploited from the seed oil, in particular when applied by obese for weight loss [1-3]. A recent review reports the impact of avocado (*Persea americana*) on distinct elements of the metabolic syndrome, such as a combination of hazard parameters, like elevated cholesterol, blood sugar, body mass index, and blood pressure. All these parameters may cause a rise in the hazard of cardiovascular diseases and type 2 diabetes.

Avocados possess the most favorable impact on lipid profiles, with alterations of HDL-cholesterol, LDL-cholesterol, triglycerides, phospholipids, and total cholesterol. However, the seed, peel, leaves, and flesh of avocados have varied impacts on elements of the metabolic syndrome [4].

The primary target of this research was to study the impact of the protein constituents separated from the aqueous extract of avocado (*Persea americana*) on biochemical specifications in an experimental animal model with the finding of an active constituent, harboring insulin-like structure or action.

Material and Methods

The crude aqueous extract preparation

Avocado (*Persea americana*) acquired from the local market in Iraq, weighing about 0.75 kg, cut in small sizes, combined with cold distilled water (DW) in a ratio of 1:3 w/v, and subsequently blended evenly for five minutes, using a mixer. The untreated, resulting material was mixed while on the ice, for another two hours, and subsequently kept in a refrigerator during the night.

Afterward, the blend was passing through some layers of filter to separate all remaining components. Ultimately, for isolating the supernatant, the mixture or the filtrate was then spun down at 8000 x g in a cold centrifuge for 15 minutes. Then, the supernatant was lyophilized in order to reduce the volume to about 1/3 for further use. The Lowry method was used to assess the total protein.

The crude aqueous extract preparation

Avocados (*Persea americana*) were acquired from the local market in Iraq, weighing about 0.75 kg, cut in small sizes, combined with cold distilled water (DW) in a ratio of 1:3 w/v, and subsequently blended evenly for five minutes, using a mixer. The untreated, resulting material was mixed while on ice, for another two hours, and subsequently kept in a refrigerator during the night.

Afterward, the blend was passed through some layers of a filter to separate all the remaining components. Ultimately, for isolating the supernatant, the mixture or the filtrate was then spun down at 8000 x g in a cold centrifuge for 15 minutes. Then, the supernatant was lyophilized in order to reduce the volume to about 1/3 for further use. The Lowry method was used to assess the total protein value.

Precipitation of the proteins

The ammonium sulfate precipitation was exploited to separate the active protein from the cold extract, and then, while stirring at 0°C, the addition of the cold crude aqueous extract was performed at a ratio of 75:100 w/v. The combination was kept in a fridge for 24 h, and then the mixture was spun down at 8000 x g for 15 minutes to separate the precipitated protein. Then, lyophilization was used to dry the precipitate. The sample was maintained at -20°C for further use.

The protein extract fractionation

About 5 ml of the concentrated sample was prepared by mixing 5 ml of distilled water with 150 mg of extract and spinning down. The gel-filtration chromatography was utilized for fractionating the protein ingredients from the plant, using a Sephadex G-75 (2.56 × 87 cm) column. The elution process was accomplished with DW.

Healthy adult rats, weighing between 150-170 g, were fasting for 16h and then randomly split into two principal sets. The first group was treated normally; meanwhile, in the second group, alloxan (125 mg/kg) was administered intra-peritoneally to induce diabetes. Each group was subsequently sub-separated into eight groups (each harboring four rats). The first set in each subgroup was served as a control set, while the rats in the other subgroups were administered intra-peritoneally the crude aqueous extract and the fractioned proteins (75 and 100 mg/kg). Following a 2-hr post-injection, and under ether anesthesia, blood samples were taken in non-heparinized micro-hematocrit capillary tubes by the orbital sinus rupture for subsequent analysis [5].

Assessment of cholesterol, total lipids, and glucose

Serum cholesterol and glucose levels were assessed based on the enzymatic techniques, using the Randox kit (UK) for glucose [6, 7]. The Chabral and Chardonnet techniques were applied for measuring the serum total lipid level [8].

Statistical analysis

Data analysis was performed using the statistical methods, including standard deviation, mean, minimum, and maximum. For comparison of diabetic and control rats, the student t-test was applied, and $P \leq 0.05$ was considered significant [9].

Results

Protein precipitation

The entire proteins from the crude aqueous extract were precipitated by the aid of the ammonium sulfate method [10]. About 54.19% of the precipitate content was protein, and the yield of the precipitation was 30.12%.

https://doi.org/10.46389/rjd-2020-1003

Fractionation of total protein

Gel filtration chromatography was employed for the fractionation, using Sephadex G75, resulting in one considerable peak, by the elution volume of 411 ml (Figure 1).

The protein level per peak was quantitatively measured after gel filtration chromatography, resulting in 15.9% and 46.1% proteins in peak A and B, respectively.

A pre-calibrated column having known protein molecular weight (MW) was used after gel filtration chromatography to determine the MM of the protein, as demonstrated in Table 1. The MW of the protein in peak B and A was estimated at 23000 and 42000 D, as shown in Figure 2.

The effects of the isolated protein component and the crude aqueous extract on glucose, total lipids, and cholesterol after intraperitoneal administration in normal rats

The crude aqueous extract and associated protein impacts on total lipids, cholesterol, and glucose, in healthy rats are shown in Table 2.

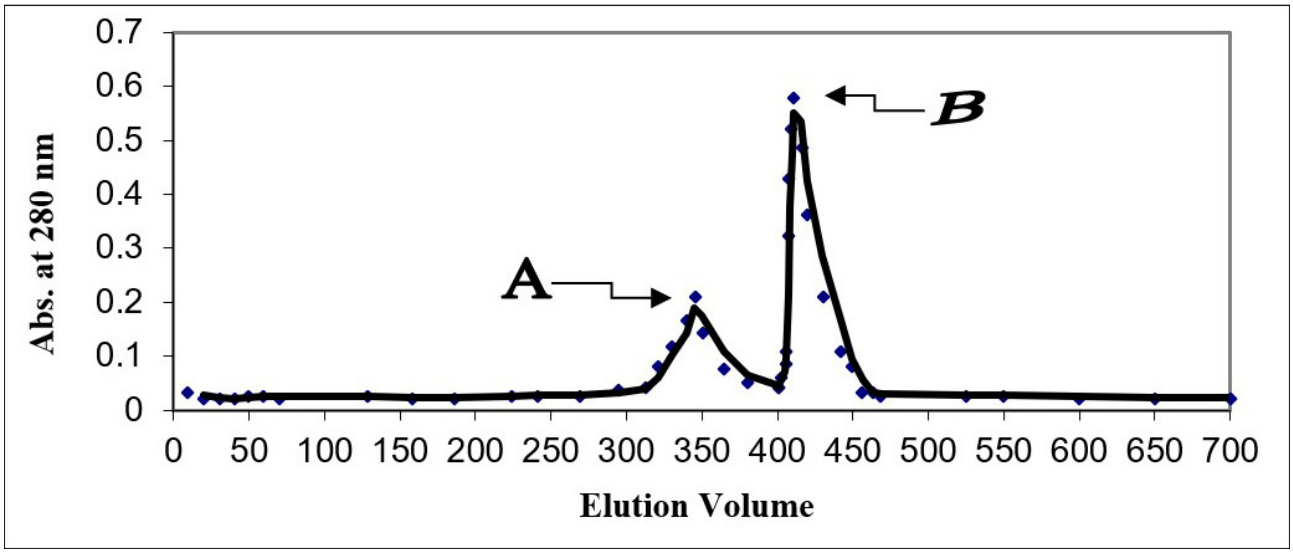

Figure 1: Elution profile of the total protein precipitate from avocado (Persea americana) - on a Sephadex G75 column (2.56 × 87cm); each fraction was eluted with 10 ml DW at a flow rate of 40 ml/h.

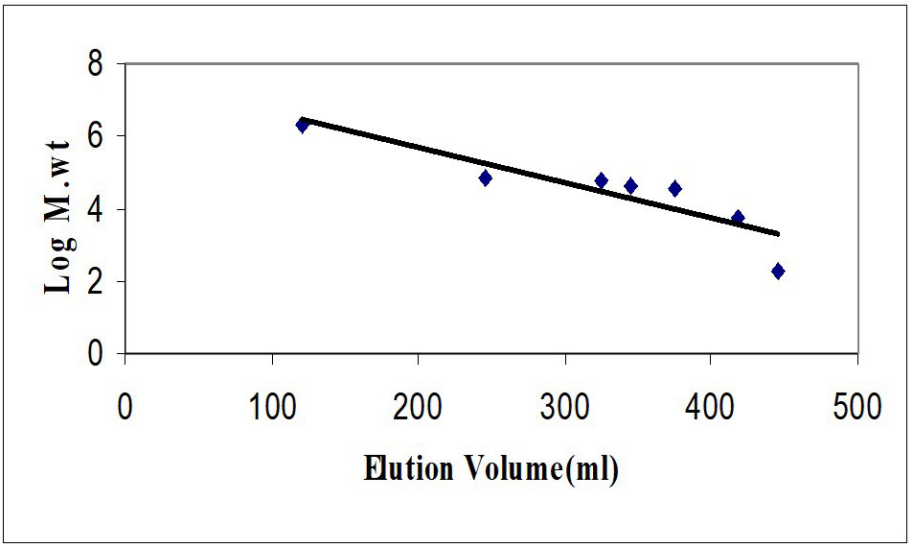

Figure 2: A logarithmic graph of elution volumes vs. MW of known proteins using a Sephadex G-75.

Table 1: Molecular weights and the associated elution volumes of separated proteins on a Sephadex G 75.

Compounds	Molecular weight (Dalton)	Elution volume (ml)
Blue dextran	2000000	121
Bovine serum albumin (BSA)	67000	246
α- amylase	58000	324
Eggs albumin	45000	345
Pepsin	36000	375
Insulin hormone	5750	418
Tryptophan	204	446

Table 2: Intraperitoneal administration of the crude aqueous extract and associated isolated protein components and the influences on serum total lipids, cholesterol and glucose, in normal rats.

Groups	Glucose (mmol/L)	Cholesterol (mmol/L)	Total lipids (mg/dL)
Control	4.70 ± 0.435	2.90 ± 0.158	487.1 ± 11.762
Crude aqueous extract	3.50 ± 0.506*	2.44 ± 0.20	403.8 ± 99.97
peak A fraction at 125 mg/kg	3.94 ± 0.19	2.65 ± 0.10	413.5 ± 78.90
peak A fraction at 100 mg/kg	3.90 ± 0.25	2.50 ± 0.13	409.5 ± 78.78
peak A fraction at 75 mg/kg	3.76 ± 0.39	2.21 ± 0.21	393.9 ± 70.30
peak A fraction at 50 mg/kg	3.75 ± 0.32	2.16 ± 0.29	383.8 ±73.53
peak B fraction at 125 mg/kg	3.76 ± 0.53	2.61 ± 0.14	377.5 ± 51.77*
peak B fraction at 100 mg/kg	3.71 ± 0.54	2.58 ± 0.17	371.5 ± 31.66**
peak B fraction at 125 mg/kg	3.76 ± 0.53	2.61 ± 0.14	377.5 ± 51.77*
peak B fraction at 100 mg/kg	3.71 ± 0.54	2.58 ± 0.17	371.5 ± 31.66**

Note: * Significant difference at P<0.05; ** Significant difference at P<0.001 .

Table 3: Effects of oral administration of the isolated protein compound and the crude aqueous extract on the lipid profile and serum glucose in normal rats.

Groups	Glucose (mmol/L)	Cholesterol (mmol/L)	Total lipids (mg/dL)
Control	4.70 ± 0.435	2.90 ± 0.158	487.1 ± 11.76
Crude aqueous extract	4.25 ± 0.33*	2.53 ± 0.26	435 ± 51.5
Peak A fraction at 125 mg/kg	4.76 ± 0.07	2.84 ± 0.16	457 ± 63.10
Peak A fraction at 100 mg/kg	4.75 ± 0.21	2.82 ± 0.19	456 ± 68.00
Peak A fraction at 75 mg/kg	4.77 ± 0.74	2.78 ± 0.16	435 ± 78.12
Peak A fraction at 50 mg/kg	4.76 ± 0.77	2.71 ± 0.17	438 ± 79.17
Peak B fraction at 125 mg/kg	4.76 ± 0.90	2.71 ± 0.13	433.4 ± 76.10
Peak B fraction at 100 mg/kg	4.67 ± 0.86	2.78 ± 0.12	438.4 ± 71.33

https://doi.org/10.46389/rjd-2020-1003

Peak B fraction at 75 mg/kg	4.74 ± 0.74	2.77 ± 0.26	391 ± 83.11*
Peak B fraction at 50 mg/kg	4.73 ± 0.86	2.73 ± 0.16	337 ± 75.19*

Note: * Significant difference at P<0.05; ** Significant difference at P<0.001.

Table 4: The influence of the isolated protein compound and the crude aqueous extract on serum total lipids, cholesterol, and glucose in diabetic rats.

Groups	Glucose (mmol/L)	Cholesterol (mmol/L)	Total lipids (mg/dL)
Control	29.6 ± 1.019	3.02 ± 0.680	657.15 ± 29.747
Crude aqueous	20.6 ± 3.65*	2.14 ± 0.43	478.51 ± 32.92*
Peak B fraction at 125 mg/kg	23.2 ± 4.13	2.37 ± 0.43	485.53 ± 15.62*
Peak B fraction at 100 mg/kg	20.5 ± 4.24	2.32 ± 0.72	487.34 ± 13.32*
Peak B fraction at 75 mg/kg	17.6 ± 8.10*	1.71 ± 0.37*	294.40 ± 32.38**
Peak B fraction at 50 mg/kg	25.6 ± 3.81	2.58 ± 0.81	473.40 ± 35.73*

Note: * Significant difference at P≤0.05; ** Significant difference at P<0.001.

After the administration of the protein fraction from peak A, no significant decline was beholden in the serum glucose in normal rats.

Oral administration of the crude aqueous extract, the isolated protein component and the effects on total lipids, cholesterol and glucose in normal rats

The data regarding the influence of protein components and the crude aqueous extract on total lipids, glucose, and cholesterol in normal rats are presented in Table 3. The data showed no significant decrease in fasting blood sugar (FBS) in normal rats due to the protein fraction (Peak A, peak B).

The intraperitoneal administration of the isolated protein compound and the crude aqueous extract, and the influences on lipid profile and glucose, in diabetic rats

The objective was to examine the impact of the protein compound of active peak and the crude aqueous extract of avocado (*Persea americana*) on serum cholesterol, total lipids, and glucose in alloxan-induced diabetic rats. Alloxan can induce diabetes by damaging the Langerhans cells, causing a reduction in the generation and secretion of insulin [4, 6]. The data regarding the impact of intraperitoneal administration on blood parameters in diabetic rats are presented in Table 4.

Discussions

Intraperitoneal administration of the crude aqueous extract and associated isolated protein components and the influences on serum total lipids, cholesterol and glucose, in normal rats

A precious decline in the FBS level was attained after intraperitoneal administration of the crude aqueous extract of avocado (*Persea americana*), compared with the control group.

A regular avocado-containing diet results in less insulin requirement. Also, when body mass index (BMI, a scale for obesity) elevates, the favorable impacts on insulin requirements, improve even further [4]. In overweight people, the ratio of C-peptide/insulin is also lower, showing that the clearance speed of insulin from the liver is increased. This is happening in addition to the insulin-lowering characteristics of avocado-containing meals, which leads to a decrease in blood sugar levels [10].

The blood level of C-peptide is an indicator of the insulin produced by the pancreas. The pancreas makes proinsulin, which is split into insulin and C-peptide and then secreted into the circulation. Therefore, a low level of C-peptide signifies that a lower amount of insulin has been produced in the blood. As a result, the level of total lipids and cholesterol decreased in the blood, in comparison with the control group. The low cho-

lesterol levels are a result of less cholesterol accumulation in the body, and high disintegration and discharge through the feces [4, 5].

Our data have also indicated that the administration of the protein component of peak B of avocado (*Persea americana*) with a dosage of 75 mg/kg, has resulted in the maximum decline (64%) in the FBS level compared to the control set, as shown in Table 2. There is a chance that the decline might be the outcome of the insulin-like activity of the protein component of avocado (*Persea americana*) [4] or due to the insulin-like shape of the protein component that attaches to insulin receptors and declines the FBS level. The protein component of peak B with a dosage of 75 mg per kilogram of body weight, presented in the same table, demonstrated a precious drop in total lipids and cholesterol. This might be the consequence of the inhibition of cholesterol production or a rise in the percentage of cholesterol excretion from the body, and the insulin-like activity may aid in lowering the serum cholesterol level [5, 11].

Avocado is a good point of supply for minerals, carotenoids, phenols, fatty acids, and vitamins. The anti-hypertensive, lipid-lowering, anti-obesity, anti-diabetic, anti-atherosclerotic, anti-thrombotic, and cardioprotective effects of avocado have been shown in other studies [4, 12, 13].

Effects of oral administration of the isolated protein compound and the crude aqueous extract on lipid profile and serum glucose in normal rats

Table 3 reports that the administration of the protein component led to a high decrease in total lipids, glucose, and cholesterol in diabetic rats, as in the case of normal rats. Nonetheless, the peak B protein compound at a dosage of 75 mg/kg was more influential in decreasing the previously mentioned characteristics in diabetic rats, in comparison to the normal rats. This declined that the impact of this investigation could help reduce the risk of hyperinsulinemia (high insulin levels in the blood), a complication related to type 2 diabetes [14, 15]. Therefore, in the diet of patients with non-insulin-dependent diabetes mellitus, the complex digestible carbohydrates can be replaced by monounsaturated fatty acids like avocado, as the principal source, which leads to the significant improvement of the lipid profile, maintenance over a suitable glycemic control, and present a proper alternative in managing the disease [14].

Some studies reported the insulin-like action of protein compounds, simplifying the entrance of glucose inside the cells, and leading to an increase in the associated metabolism [16-18].

Following oral administration of the protein fractions (Peak A and B) and the crude aqueous extract of avocado (*Persea americana*), an increase in the serum glucose was found. This might be due to the constituents such as proteins, polysaccharides, amino acid, and fats in the crude aqueous extract. As glycogenolysis, glycolysis, and gluconeogenesis become active, these compounds, after metabolic reactions, increase serum glucose. An increase in the serum glucose was also found after oral administration of the protein fraction, Peak A and peak B; the proteins may be inactivated by the proteolytic enzymes or broken down by the gastric juice.

Taken all together, the intraperitoneal administration of the protein fraction and the plant extract have a significant hypoglycemic effect compared to the oral administration, and it is the most recommended path.

The influence of the isolated protein compound and the crude aqueous extract on serum total lipids, cholesterol, and glucose in diabetic rats

As presented in Table 4, the administration of the protein component led to a high decrease in total lipids, glucose, and cholesterol in diabetic rats, as in the case of normal rats. Nonetheless, the peak B protein compound with a dosage of 75 mg/kg was more influential in decreasing the above-mentioned characteristics in diabetic rats, in comparison to the normal rats. This lowering impact of this investigation could contribute to the reduction of the risk of hyperinsulinemia (high insulin in the blood), a complication related to type 2 diabetes [14, 15]. Therefore, in the diet of patients with non-insulin-dependent diabetes mellitus, the complex digestible carbohydrates can be replaced by monounsaturated fatty acids like avocado, as the principal source, which leads to the significant improvement of the lipid profile, maintenance over a suitable glycemic control, and present a proper alternative in managing the disease [14]. Some studies reported the insulin-like action of protein compound, simplifying the entrance of glucose inside the cells, and leading to an increase in the associated metabolism [16-18].

Conclusion

In terms of novelty, this study showed that avocado (*Persea americana*) has the potential to be used for di-

 https://doi.org/10.46389/rjd-2020-1003

abetes therapy due to active protein components. Furthermore, the glucose and lipid ratio was smaller after having an avocado, demonstrating high liver potency in insulin clearance. Avocado also has antioxidants, such as carotenoids and vitamin C, which might contribute to the insulin regulation, which is applicable in reducing the blood sugar and lipids.

Acknowledgment

The author would like to thank the deanship of the College of Science and the Presidency of the University of Mosul for supporting this research.

Conflict of Interest

The author declares no conflict of interest.

References

1. Lopez Ledesma R, Frati Munari AC, Hernandez Dominguez BC et al. Monounsaturated fatty acid (avocado) rich diet for mild hypercholesterolemia. Arch Med Res 27(4): 519-23, 1996.
2. Alhassan AJ, Atiku M, Sule MS, Wudil AM. Effects of aqueous avocado pear (Persea americana) seed extract on alloxan induced diabetes rats. Greener Journal of Medical Sciences 2(1): 005-011, 2012.
3. Roger CR. The nutritional incidence of flavonoids: some physiological and metabolic considerations. Experientia 44(9): 725-33, 1988.
4. Tabeshpour J, Razavi BM, Hosseinzadeh H. Effects of Avocado (Persea americana) on Metabolic Syndrome: A Comprehensive Systematic Review. Phytother Res 31(6): 819-837, 2017.
5. Aljawadi ZAM. Isolation and Studying the Effect of Protein Fractions of Red chili pepper (Capsicum annuum L.) on Some Biochemical Parameters in Experimental Diabetic Rats. International Journal of Enhanced Research in Science. Technology & Engineering 7(2): 51-56, 2018.
6. Passing H, Bablok A. New biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry. Part I. J Clin Chem Clin Biochem 21(11): 709-20, 1983.
7. Tietz NW. Text book of Clinical Chemistry. 3rd ed. 1999: C.A. Burtis, E.R. Ashwood, W.B. Saunders. pp: 809-856.
8. Aljawadi ZAM. Isolation and Studying the Effect of Protein Fractions of White Cabbage (Brassica oleracea var. capitata) on Some Biochemical Parameters in Experimental Diabetic Rats. Int Res J Med Sci1(11): 17-22, 2013.
9. Torrie J, Steel R. Principles and Procedures of Statistics a Biometrical Approach. 2nd ed. Singapore: McGraw-Hill Inc, 1986
10. Mohamed M, Abdel N, Rashad M. Hypoglycemic and Hypocholesterolemia Effects of Intragastric Administration of Dried Red Chili Pepper (Capsicum Annum) in Alloxan-Induced Diabetic Male Albino Rats Fed with High-Fat-Diet. Journal of Food and Nutrition Research 2(11): 850-856, 2014.
11. Esayas K, Shimelis A, Ashebir F, Negussie R, Berhanu T, Gulelat D. Proximate composition, mineral content and antinutritional factors of some capsicum (Capsicum annum) varieties grown in Ethiopia. Bulletin of the Chemical Society of Ethiopia 25(3), 2011.
12. Mohiuddin AK. Natural Foods and Indian herbs of cardiovascular interest. Pharmacy & Pharmacology International Journal 7(2), 2019.
13. Wang L, Bordi PL, Flemig JA, Hill AM, Kris-Etherton PM. Effect of a moderate fat diet with and without avocados on lipoprotein particle number, size and subclasses in overweight and obese adults: a randomized, controlled trial. J Am Heart Assoc 4(1): e001355, 2015.
14. Aslan M, Orhan N, Orhan DD, Ergun F. Hypoglycemic activity and antioxidant potential of some medicinal plants traditionally used in Turkey for diabetes. J Ethnopharmacol 128(2): 384-9, 2010.
15. Liang YT, Tian XY, Chen JN et al. Capsaicinoids lower plasma cholesterol and improve endothelial function in hamsters. Eur J Nutr 52(1): 379-88, 2013.
16. Lerman-Garber I, Ichazo-Cerro S, Zamora-Gonzales J, Cardoso-Saldana G, Posadas-Romero C. Effect of a high-monounsaturated fat diet enriched with avocado in NIDDM patients. Diabetes Care 17(4): 311-5, 1994.
17. Ozgur M, Ozcan T, Akpinar-Bayizit A, Yilmaz-Ersan L. Functional compounds and antioxidant properties of dried green and red peppers. African Journal of Agricultural Research 6(25): 5638-44 , 2011.
18. Chandini R, Binoy JP, Udayabhaskaran V, Ramamoorthy KP. A study of non-obese diabetes mellitus in adults in a tertiary care hospital in Kerala, India. International Journal of Diabetes in Developing Countries 33(2): 83-85, 2013.