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Nutritional Evaluation on *Stevia Rebaudiana* Leaves by Chemical, Biological and Microbiological Studies

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Abstract

Stevia rebaudiana is a natural herb of Asteraceae family containing zero calorie or non- nutritional sweet compounds. It has therapeutic, antioxidant, and microbial properties. This study aimed to identify chemical composition; amino acid, sugar, energy contents and some microbial and biological properties of stevia leaves. The results showed that protein, fat, ash, Carbohydrates (CHO) and fiber contents of stevia leaves were (8.06, 4.2, 7.52, 69.52, and 10,7 g/100gm dry matter) respectively. The estimated caloric content was 4.22 Kcal / g stevia leaves. Mineral contents were determined (mg/100 g) dried stevia leaves and recorded as: Ca (190), P (198), Na (135.8), K (1799), Mg (598) and Zn (4.2). Reduced sugar contents in stevia leaves were found to be 0.852, 0.488 and 1.66 g/kg leaves for sucrose, glucose and fructose respectively. Total reduced sugar was 0.3%. The biological experiment on hypercholesteremic rats revealed that the consumption of aqueous stevia extract has significantly reduced the levels of cholesterol, triglyceride, Low Density Lipoprotein (LDL-C), while it noticed an increased in High density Lipoprotein (HDL-C) was noted which is desirable. Thus it is concluded that stevia extract has hypolipidemic effect and can be used to reduce the risk of Cardio Vascular Diseases (CVD). The microbiological study showed that 1% (wt/vol) effective extract of stevia leaves lead to decrease of *Salmonella typhimurium* count and increase lactic acid bacteria count. On the other hand, it was noticed that ethanolic extract of stevia leaves was more effective against *S. typhimurium* than aqueous extract. The study concluded that stevia can be used as therapeutic diet and preservative.

Keywords: Biological; Biochemical; Chemical Analysis; Jam Processing; Lipidemic; Microbial Analysis; *Stevia Rebaudiana*

Introduction

In Egypt, according to annual population increases, a persistent need to decrease the gap between the production and consumption; consequently, Foreign Agriculture Service in United State Department of Agriculture (USDA) reported that Egyptian domestic sugar consumption must be increased by 100.000 Million Ton (MT) □3.4% to reached from 2.950 to 3.05 MT in Marketing Year (MY) 2016/017 to 2017/018 respectively [1].

The first objective to achieve Egyptian Agriculture strategy 2030 edited by Abaza, 2009 [2] "Sustainable use of Agriculture Natural resource" and the first sub objective was "Enhancing water- use efficiency in irrigated agriculture" is searching for new crops to maintain water. The average irrigation water needed for beet sugar [3], cane sugar [4] and stevia [5] were 2500-3000 (Km^3/Feddan/7months), 7500-10000(Km^3/Feddan/12 months) and 4000- 5000(Km^3/Feddan/12 months) respectively.

Stevia rebaudiana Bertoni Stevia leaves, is natural sweetener essence plant of 200 species of genus Stevia (Savita et al., 2004) [6] family of Asteriaceae (sunflower), often referred to as ''the sweet herb of Paraguay'' [7-9]. It is a white, crystalline powder having commercial properoities, with some other sweet compounds such as steviobioside, rebaudioside A, B, C, D, E and dulcoside A, the presence of high concentration of some essential oils tannins and flavonoids are responsible for bitter after taste [10].

One kilogram of Stevia is around 200 times sweeter than one kilogram of granular sugar, stevia provides zero calories; it is heat stable up to198 ˚C, non-fermentable, a taste enhancer, helpful in digestion, weight reducing agent, antioxidant, antimicrobial and is anti-plaque properties, increases mental alertness, increase energy levels but does not affect the blood sugar level therefore it has hypoglycemic effect. Stevioside has an effect for intensifying insulin sensitivity with fructose [11-13]. Stevia can be used in hypertension, helpful in skin toning, healing, tobacco and alcohol

cravings and a tonic for pancreas and anti-caries [14,15], It can also be used as alternative source of sugar for food confectioneries, bakeries, fruit juices, jams, biscuits, chocolates, vegetables and other food stuffs. Consumer trends to prefer foods derived from natural resources that lack artificial components such as aspartame and saccharine, in which reported of some neuropsychiatric disorders, headache and dermatologic reactions [16].

Jayaraman et al., (2008) [17] proved that 50 mg/ml of stevia leaf extracts (ethyl acetate, acetone and chloroform) had antibacterial, anti-yeast, and antifungal activity e.g. *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Vibrio cholera, Candida albicans, Cryptococcus neoformans, Trichophyton mentagrophytes,* and *Epidermophyton*.

Debnath (2008) [18] reported that Crude leaf methanolic and chloroform extracts were the most effective than water extracts against S. aureus followed by *Staphylococcus mutans*, *B. subtilis* and *E. coli* respectively on the basis of the zone of inhibition (MIC). On the other hands, maximum zone of inhibition was shown by *Sclerotinia minor*. The antimicrobial and antifungal effect was similar to tested antibiotics; those effect due to formation of secondary metabolites responsible for antimicrobial effect.

The aim of this study is to characterize some chemical contents and antibacterial properties of stevia leaves, to evaluate the biological effects on lipid profile, and to compare sensory differences between 3 types of sugar (sucrose, fructose and stevia) used in jams.

Materials and Methods

Chemical Composition

a) Raw Materials

Stevia leaves obtained from Sugar Crops Institute - Agriculture Research center - Giza, Egypt. The proximate, chemical, microbiological, biological, biochemical analysis have been done in the Regional center for Food and Feed- Agriculture Research Center- Giza, Egypt. Viscosity have been determined on National institute for food processing.

b) Chemical analysis

Stevia leaves was oven dried on 60º C overnight, until constant weight. Proximate analysis including moisture, total protein, fat, ash, minerals and crude fiber were carried out according to [19]. Carbohydrate content was calculated by difference. The fatty acids content was measured according to standard fatty acids methyl esters (FAME) [20] using Gas-Liquid Chromatography (GLC) technique. Minerals determination was carried out using Optima 2000DV inductively coupled plasma spectrometer with full PC control (Perkin Elmer). Concentrations were obtained based on calibration curves developed by using (Merck) ICP standards.

Amino acids determination was performed according to (AOAC, 2007) [19] using amino acids analyzer (Biochrom 30) through ion exchange resin via ninhydrin post-column derivatization.

c) Reducing sugar

Reduced sugars content were determined according to ICUMSA (1998) [21] using 4 g of stevia leaf powder. The sample was placed in a 200 mL flask, and the volume made up to mark with distilled water. The mixture was filtered using a What man (no. 1) filter paper and titrated with Fehling's solution to determine reducing sugars content.

d) Estimated energy

Laboratory determination of energy was carried out according to operating instruction manual for [22].

e) Caloric energy value

Energy was determined according to the method described by Atwater, & Benedict, (1902) [23]. By this determination, 1 g of carbohydrate provides 4 kcal; 1 g of protein provides 4 kcal and 1 g fat provides 9 kcal.

Biological Experiment

The preparation of stevia extract was standardized Through sensory evaluation, considering 66 g of stevia leaf powder was boiled with 1500 ml water for 3 hours and 20 minutes, which produced 400 ml of extract. which have been prepared the freshly in the early morning every day. The five weeks old male albino rats were individually housed in stainless steel cages and maintained at 22-24 ˚C of relative humidity 45-55%. Diet and water were provided *ad-libitum*. Adaptation time took three days using barley as the sole diet. All diets were analyzed for moisture, crude protein, fiber, fat and ash according to [19].

Eighteen rats were randomly distributed on three groups: Group 1 served as negative control fed on normal diet (a) which was formulated according to (NRC, 1995) [24] and drunk tap water. Group 2 " positive control" fed on high fat diet (b) included 20% soya bean oil as fat source to prepare high fat diet and drunk tap water. Group 3 fed on high fat diet (b) and supplied with freshly prepared aqueous-stevia extract. At zero time, after 4 and 8 weeks (two months); blood samples were collected centrifuged at 3000 rpm for 15 min.

Serum was separated and kept at 4 ˚C until biochemical analysis of triglycerides, cholesterol [25], High Density Lipoprotein (HDL) (Friedewald et al., 1972) [26] and Low Density Lipoprotein (LDL) [27].

Microbiological Evaluation

a) Bacteria strains

Pathogenic bacteria strain i.e. *Salmonella typhimurium* was

used to evaluate the effect of natural antimicrobial agents. This strain was kindly provided by Abdel- Salam, A.F., Regional Center for Food and Feed, Agriculture Research Centre (ARC), Giza-Egypt. *Salmonella typhimurium* isolates were maintained through monthly transfers on nutrient agar and stored at 4 ˚C.

b) Standard inoculum

Standard inoculum was prepared by inoculation conical flask (100 mL in volume containing 50 mL of 1% buffered peptone water (pH 7.2) for 24 hr. at 37 ˚C with loop of *S. typhimurium*. Viable cells counts were determined by serial dilution and subsequent enumeration on S.S agar.

c) Effect of stevia leaves on growth of *S. typhimurium in vitro*

Erlenmeyer flasks 250 mL contained 50 mL of 1% buffered peptone water were inoculated with 1 mL of *S. typhimurium* containing about 108 cfu/mL, then added concentrate 1% of stevia leaves (powder and ethanolic extract) to each flask separately. The flasks were incubated at 37 ˚C for 24 hours on rotary shaker (100rpm). The control was inoculated with *S. typhimurium* only.

d) Effect of stevia leaves on growth of lactic acid bacteria in yoghurt

Stevia leaves ethanolic and aqueous 1% solution were added to yoghurt samples. The controls were left without any treatment. Yoghurt samples were incubated at 37 ˚C for 3 hours, then put at 4 ˚C and the mean cfu/g was determined for lactic acid bacteria according to [28].

e) Antimicrobial assay using the diffusion method

The petri plates were poured with 20 ml of nutrient agar, after inoculated it with microbial culture of *S. typhimurium*. The microorganism and growth medium were mixed thoroughly to ensure uniform distribution of the microorganism. Sterilized filter discs (whatman type1, 0.6 cm in diameter) were placed on the surface of nutrient agar. Each aqueous and ethanolic extract was tested by addition 100 ml of each type to each filter paper disc. Plates for antimicrobial activity test were incubated at 37 ˚C for 24 hours. The experiment was done 3 times. The inhibition zone diameter was measured using Vernier calipers and expressed in millimeters.

Pumpkin Jam

Three types of pumpkin jams have been cooked with crystal sugars sucrose, fructose and stevia (purchased from local market). 750 gm of pumpkin fruits cut in big portions and divided in 3 cooking pots then adding 1 gm of stevia, 150 gm fructose and 250 gm of sucrose into each pot separately and kept boil till tender. 150 gm of mashed boiled green apple as source of pectin and 1gm lemon salt was added to each pot to make jam.

The amount of fructose was equivalent of 1.5 times sweeten respectively from sucrose Armand Cardello et al., 1979 [29], while stevia was equivalent of 200 times sweeten from sucrose (Chang et al., 2005) [11], which considered as standard consumed sugar which have sweetener rate 100%.

Organoleptic Evaluation

Organoleptic evaluation of the three jam samples was conducted as described by Iwe (2002) [30] using 30 members panel randomly selected from the area of Regional center for food and feed community. The samples were packed in a transparent jam bottled and presented in a coded letter. The sensory quality attributes of the samples were color, taste, aroma, texture, sweetness, palatability and notes.

The questionnaire sheet presented to the panelists, they were requested to fill the panelists according to the grade based on a 3 points showing high, moderate and least acceptable in all attributes. The test was also provided with potable water to rinse their mouth after evaluating each sample to avoid taste interference. The samples were also photocopied using digital camera.

Viscosity Evaluation

Viscosity was determined in Food Technology Research Institute, Agriculture Research Center Giza, Egypt, using Brookfield Viscometer DVIII utra (Rpm 10, Temperature 25 ˚C, spindle No. HA-07).

Statistical Analysis

Analysis of variance (Multivariate) and Duncan's test were conducted using a Statistical Analyses software [31]. A probability to ($P \leq 0.05$) was used to establish the statistical significance. The sensory evaluation was done using one factor analysis of variance

Results and Discussions

Chemical Composition

The chemical composition of dried stevia leaves has been studied in previous researchers, the chemical analysis for 100gm dry matter of current study is presented in table 1, protein contents was 8.06 gm/100gm DM of stevia leaves, it was lower than [32-

34] which ranged from 11.2 - 16.23 g/ 100 gm DM stevia. Fat contents was 4.2 g/ 100gm DM stevia similar to [34-35]. Ash content was 7.52 g/100gm DM stevia leaves it was in the similar with [33]. Carbohydrate content was 69.52 gm/100gm DM of stevia leaves in the near range 62 g/ 100gm DM stevia leaves to [33].

Chemical composition	Protein	Fats	Ash	Carbohydrate	Dietary fiber
Value (gm/ 100gm) DM	8.06	4.20	7.52	69.52	10.70

Table 1: Chemical composition of Stevia leaves gm/100gm dry matter basis (DM).

Mineral contents

Data in table 2 revealed that mineral contents mg/100gm of dried stevia leaves were high in Calcium, Magnesium, Potassium and Zinc contents. Khiraoui A. et al.; (2017) [34] studied variable breeding on different sex cultivated areas (from sandy to silty clay soils and from arid to sub humid bio-climate) in Morocco; mineral composition showed very wide differences in analyzed results for Ca (579.5: 733.7), Na (69:190), K (1633:2787), Mg (179.6:198) Zn (1.71:5.32) mg/100gm of stevia leaves. Na and P values were near to [6] 190 and 1800 mg/100gm DM stevia leaves respectively.

Minerals	Calcium	Phosphorus	Sodium	Potassium	Magnesium	Zinc
Values	190	198	135.8	1799	598	4.2

Table 2: Contents of minerals in dried leaves of Stevia mg·100/ g.

Amino acid contents

Essential Amino acids	Contents	Non- Essential Amino acids	Contents
Threonine	0.37	Alanine	0.4
Valine	0.42	Aspertic	0.65
Iso- Leucine	0.36	Serine	0.33
Leucine	0.6	Glutamic	0.77
Phenyl Alanine	0.39	Glycine	0.37
Histidine	0.18	Proleine	0.92
Argenine	0.39	Tyrosine	0.29
Lysine	0.48	Cysteine	0.58
Methionine	0.99	-	-
Tryptophan	ND	-	-
Total	**4.18**	**Total**	**4.31**

Table 3: Amino acid contents for stevia leaves mg/ 100 g protein.

The amount of essential amino acids was similar to non-essential amino acids in current study. Leucine and methionine were the highest essential amino acids (0.6 and 0.99 mg/100gm protein) respectively; which agreed with Li et al., (2011) [36] who reported that leucine was the highest essential amino acid 1.300 mg/100gm protein, noticed that he didn't analyze methionine; as thus proleine and glutamic were the highest non- essential amino acids (0.92 and 0.77 mg/100gm protein) respectively.

On contrast; the lowest essential amino acid was Histidine (0.18) mg/100gm protein and non- essential amino acid was tyrosine (0.29) mg/100gm protein this agreed with [36] proved that there is a wide range for amino acid contents in his study, as the same Proline and tyrosine were the highest and the lowest non-essential amino acid 1.729 and 0.495mg/100gm protein respectively.

Li et al., (2011) [36] analyzed amino acids contents using wide varieties of stevia leaves, his essential amino acid contents was 5.89 near with current study 4.18 mg/100gm protein. Non-Essential amino acid contents in the current study were nearly the same figures with the study conducted by [33] 3.82 compared with 4.31 mg/100gm protein in the current study.

Sugar Contents

Sweet components vary between 4- 20% of the dry weight of the leaves depending on stevia genotypes, treatments, pH and growing conditions [37]. The sugar contents in stevia leaves determined to be 0.852, 0.488 and 1.66 g/kg leaves for sucrose, glucose and fructose respectively, the total reduced sugar was 0.3%, agreed with [33,38] which determined reducing sugar ranged from 0.45-5.3 and 0.3-0.36 %. Gasmalla et al., (2014) and Khiraoui et al., (2017) [34,38] reported that reduced sugar contents ranged from 3.87 to 5.876 gm/ 100 gm dry weight basis; with the average of 4.83 which is the same of our result (4.88) gm/ 100 gm dry weight basis. The sweeteners sugars of stevia were sucrose (8.52) and fructose (16.6) gm/100 gm dry matter basis.

Caloric Measurements

The estimated and calculated calories for stevia dried leaves presented in table 4 they were 422 and 348 Kcal/100 gm respectively; likewise, [34,38] who calculated energy contents ranged 362.3 - 384.2 kcal/100 gm. The estimated calories measure gross energy which defined as "the total chemical energy measured from complete combustion of the food in a bomb calorimeter" [39]. Calculated energy depend on Metabolic Energy (ME) which defined as "the amount of energy available for total (whole body) heat production at nitrogen and energy balance" Livesey, 2001 [40] and it was calculated using Atwater factor. There are many theories to explain these differences. The most reasonable explanation that according to definition they are two different types of energy. The other explanation going through that foods that provide substantial amounts of energy as protein, fat and carbohydrate in the ordinary diet, energy conversion factors in the Atwater specific factor system varies. These ranges are respectively, 44, 7 and 35 percent [41]. The last explanation that Atwater specific factor system appears to be take only protein, fat and total carbohydrate into account, and neglect the differentiation between available carbohydrate and dietary fiber into account.

Caloric contents (Kcal/100 gm)	Calculated	Estimated
	348	422

Table 4: Caloric contents in dried stevia leaves on dry matter basis (Kcal/g).

Finally; it could be concluded that chemical composition for stevia leaves started in Japan by [42] till now, many researches [32-36,43,44] studied chemical composition, mineral, amino acid and sugar contents, in many geographical areas and countries with different environmental condition from Japan, Morocco, Turkey, Iran, Al- Salvador and Egypt, that results had wide range of composition which could be reflecting to geographical and genotypes variations.

Microbial Results

The results in table 5 clearly showed that stevia (powder and ethanolic extract) at concentrate 1% lead to decreasing of *S. typhimurium* counts from 6×10^9 to 9×10^5 and 3×10^5 cfu/ml respectively; on the other hand, increased Lactic acid bacteria counts from 3×10^3 to 8×10^5 and 6×10^6 cfu/ml at concentrate 1% of powder and ethanolic extract respectively. Data reported in table 6 revealed that the ethanolic extract of stevia leaves was more sensitively against *S. typhimurium* than aqueous extract, such exhibited higher diameter of inhibition zone against *S. typhimurium* in 15mm, while aqueous extract recorded inhibition zone in 6mm against *S. typhimurium*. The results were agreement with those recorded by [45] who examined the antimicrobial activity of organic extracts from stevia and stevioside against a wide spectrum of food-borne pathogens, including *B. subtilis, Bacillus cereus, Staphylococcus xylosus, Alcaligenes denitrificans, Kleb siella pneumoniae, Pseudomona aeruginosa, Salmonella typhimurium*, and *Serratia marcescens*. The extracts were tested at two concentrations, 500 and 1000 µg/mL, and displayed a dose-dependent inhibition across all the microorganisms tested. Ethanol and acetone extracts displayed the broader spectra of inhibitory activity.

Treatments / Microorganisms	Powder 1%	Ethanolic extract 1%	Control
S. typhimurium	9×10^5	3×10^5	6×10^9
L.A.B.	8×10^5	6×10^6	3×10^3
The used inoculum of *S. typhimurium* was 5×10^8 cfu/ml			

Table 5: Effect of stevia leaves on growth of *S. typhimurium* in vitro and lactic acid bacteria in yoghurt (cfu/ml).

Treatment / Microorganism	Aqueous	Ethanolic extract
S. typhimurium	6	15

Table 6: Zone of inhibition (mm) of stevia leaves against *S. typhimurium*.

Biological Study

Analysis of variance on the effect of feeding rats with aqueous stevia extract on serum lipid profile was presented in table 7; which proved an improvement of mean HDL value compared with both +ve control (high fat group) and -ve control "normal fat" (93.77 versus 64.33 mg/dL respectively), with highly differences ($P < 0.05$). On the opposite, the mean values LDL was decreased in G3 compare with positive control group followed by negative control LDL (64.33 versus 90.83 mg/dL), triglycerides (59.64 versus 68.72 mg/dL) and total cholesterol (118.65 versus 140.45 mg/dL). Furthermore, administration with stevia showed very high significant reduction for LDL, triglyceride and total cholesterol (P

<0.05); this agree with sharma et al., (2009) [46] who reported a reduction on LDL, triglyceride and total cholesterol while HDL increased for hypercholesteremic women after intervention with stevia aqueous extract. Many studies proved the ability of stevioside in lowering blood cholesterol which increase the bile acid excretion by preventing re-absorption from small intestine through disturb of micelle formation of acid bile. The increase in excretion of bile acid and cholesterol 7-α hydroxylase enhances the diversion of liver cholesterol to bile acid resulting in cholesterol reduction Ahmed et al., (2018). Current study proved that triglyceride decreased with group administrated with aqueous stevia extract than control positive group (high fat intake) with mean values (58.64 versus 68.72 ml/dL) respectively. Ahmed et al., (2018) discussed many mechanisms in reducing triglyceride after administration with aqueous extract of stevia, one of them is referred to glucoside as a major substance in stevioside which enhance excretion of hepatic lipase enzyme resulted on lipid degradation. Other mechanisms are decreasing dietary lipid absorption in the intestine through reducing micellar solubilisation of cholesterol and increase excretion of triglyceride in faces. The last mechanism is due to the inhibition role of stevioside on activating acetyl- coenzyme A carboxylase and fatty synthase.

Parameters / Groups	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)	Total cholesterol (mg/dL)
G1 "C-"	84.72^{b} ±2.038	68.55^{b} ±1.90	62.13^{ab} ±1.78	124.144^{b} ±1.86
G2 "C+"	64.33^{c} ±2.13	90.83^{a} ±2.30	68.72^{a} ±2.90	140.155^{a} ±4.81
G3 "Stevia"	93.77^{a} ±3.40	64.33^{b} ±2.60	58.64^{b} ±1.16	118.650^{b} ±1.69
Within the same column, various superscript letters indicate significant differences (Duncan, $P < 0.05$).				

Table 7: The effect of aqueous stevia extract on serum lipid parameters at the end of experiment (means ± SE).

Parameters	Time	G1 "C-"	G2 "C+"	G3 "Stevia"
HDL- Cholesterol (mg/dL)	**0 week**	74.8^{d}±0.7	74.8^{d}±0.7	74.8^{d}±0.7
	4 week	85.8^{c}±1.3	63.5^{e}±1.0	99.5^{d}±1.1
	8 week	94.2^{b}±1.5	54.7^{f}±2	107^{a}±1.7
LDL- Cholesterol (mg/dL)	**0 week**	78.5^{b}±0.9	78.5^{b}±0.9	78.5^{b}±0.9
	4 week	66.3^{c}±1.5	94.2^{a}±1.5	60.3^{c}±1.4
	8 week	60.8^{c}±1	99.2^{a}±2	54.2^{d}±1.5
Triglyceride (mg/dL)	**0 week**	53.8^{d}±0.5	53.8^{d}±0.5	53.8^{d}±0.5
	4 week	62.13^{c}±2	69.5^{b}±1	57.5^{d}±0.8
	8 week	70.43^{b}±0.75	82.8^{a}±0.7	64.5^{c}±1
Total Cholesterol (mg/dL)	**0 week**	117.1^{de}±2.3	117.1^{de}±2.3	117.1^{de}±2.3
	4 week	123.5^{de}±1.8	140^{b}±3	113^{e}±2.3
	8 week	131.8^{bc}±2.2	163.3^{a}±3	125.8^{cd}±1.3
Within the same column, various superscript letters indicate significant differences (Duncan, $P < 0.05$).				

Table 8: The effect of Time with groups treatments on serum lipid parameters (means ± SE).

The data on Table 8 presents the changes on lipid parameters as indicator of each groups during experiment periods; the mean values ± SE of HDL have been increased by time from mean value of 74.8(mg/dL) at the beginning of the experiment to mean value of 107(mg/dL); also, it showed the highest increment when compared with positive and negative groups figure 1 while LDL mean values decreased by time from 78.5 to 54.2 mg/dL at zero till 8 weeks respectively, the use of stevia "G3" also was lower than control+ group (high fat intake) which recorded the highest value of LDL 99 mg/dL figures 2-4. Occasionally, occurred with the mean values of triglycerides and total cholesterol were the highest in control + group during the time. General linear analysis showed very high significant correlation P<0.05 for mean values of HDL, LDL, triglyceride and total cholesterol within groups during experiment periods. The percentage of lipid parameter reduction after administration with stevia compared with high fed intake (positive group) was agreed with Ahmed et al., (2018) who fed his hyperlipidemic rats (which induced by mixing 400 mg cholesterol

90%E /kg body weight rats for 8 weeks + aqueous stevia extract on the percentage of reducing total cholesterol (15.34%- 16.8% respectively),otherwise the current study present improvement by 45.75% in HDL and also in reducing of triglyceride by 14.70% when comparing G3 "high fat diet with stevia aqueous extract" with G2 "high fat diet with distilled water.

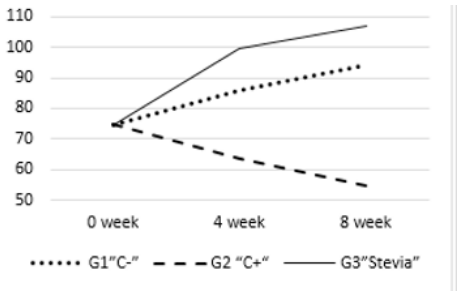

Figure 1: HDL m(g/dL) changes during experiment treatments.

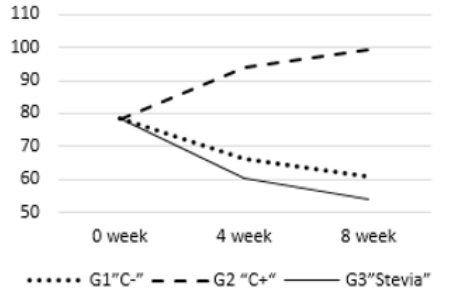

Figure 2: LDLm(g/dL) changes during experiment treatment.

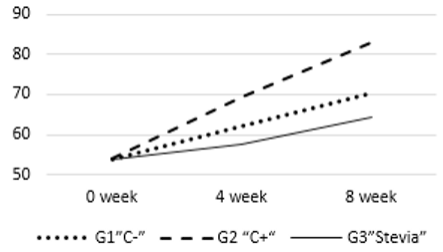

Figure 3: Triglycride (mg/dL) changes during experiment treatment.

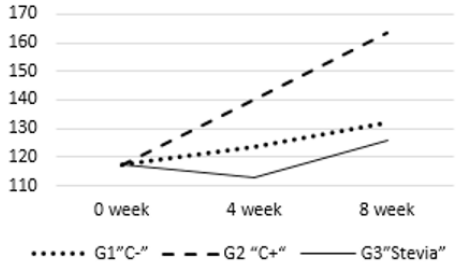

Figure 4: Total cholesterol (mg/dL) changes during experiment treatments.

Pumpkin jam with different types of sugar

Table 9 present the quantity of three types of sugar (sucrose, fructose and stevia) was added to pumpkin jams; energy contents and viscosity. Descending caloric energy contents for stevia, fructose and sucrose pumpkin jam were 252.5, 308.7 and 362 Kcal/ 100gm edible jam respectively. The small quantity of stevia added to pumpkin jam (0.4gm/ 100 gm pumpkin jam) refer to the high sweetness for stevia leaves 300 times compared with fructose and sucrose (60 and 74.8 gm/100gm pumpkin jam respectively); which present the importance of use stevia in therapeutic nutrition and food processing manufacturing. Current research reported highest viscosity value was stevia followed by sucrose and fructose (111200, 102400 and 86400 CP respectively). Norazlanshah Hazali et al., (2014) [47] proved that after short period consumption (30 min) of 500 mg from sucrose and stevia for 18 to 23 year adults, blood sugar has been decreased with stevia compared with sucrose consumption. The author recommended to consume stevia maintain blood glucose even when consumed in short length of time.

Types of sugar	Sucrose	Fructose	Stevia
Quantity of sugar (gm) added to (100gm) jam	74.8	60	0.4
Energy content Kcal /100gm edible jam	362	308.7	252.5
Viscosity (CP)	102400	86400	111200

Table 9: Quantity, estimated caloric contents and viscosity of sugar added to pumpkin jam with 3 different types of sugars.

Organoleptic Evaluation

The results of rank method for the organoleptic evaluation of the pumpkin jam color, taste, texture sweeter and overall acceptability are shown in table 10. From these data, it could be noticed that sucrose jam was the best choice of all parameters followed by fructose jam, while stevia showed less acceptance for all studied characteristics. There are very high significant different between sucrose, fructose and stevia in studied parameters ($P<0.05$).

Type of jams	Sucrose	Fructose	Stevia
Rank of jams as advantage*			
Color	1^a	2^b	3^c
Taste	1^a	2^b	3^c
Texture	1^a	2^b	3^c
Sweeter	1^a	2^b	3^c
Overall acceptability	1^a	2^b	3^c
*The numbers mean rank, 1= highly accepted, 2= mediate acceptance, 3= less accepted. ^{a,b,c,} means a raw followed by the same letter are non-significantly different **(P<0.05)**			

Table 10: Rank method for organoleptic evaluation.

Table 11 present the ranking of overall acceptability for tested jams with different levels of significant P< 0.05 and P<0.01. Overall acceptability statistical result showed very high significant differences between uses of sucrose, fructose and stevia. Sucrose was the highest accepted followed by fructose, otherwise stevia was not accepted. Small stevia quantity added (0.4gm/ 100 gm pumpkin jam) didn't allow to produce sugar syrup and resulted puree product. Therefore; it could be concluded that, stevia is preferable to be used as hidden sugar like in juices, desserts, biscuits, cake, heavy sauces, chocolates and puree.

Type of jams	Sucrose	Fructose	Stevia
Rank sum	20	40	60
Difference vs.			
Sucrose		20	40
Fructose			20
Significant levels	**P < 0.05**		**P < 0.01**
Critical differences	15.00		18.4
Product × Rank			
Sucrose	a		a
Fructose	b		b
Stevia	c		c
a,b,c, means a raw followed by the same letter are non-significantly different (P<0.05, P<0.01)			

Table 11: Rank method for overall acceptability.

Mondaca et al., (2012) [48] reported that Food Drug Administration (FDA) in Dec./ 2008 and France in August/ 2009 stated the purified stevia considered as safe in use to sweeten beverages and some foods. Therefore, the Joint FAO/WHO expert committee (2008) on food additives (JECFA) suggested a temporary Admissible Daily Intake (ADI) of 0-4 mg kg/BW of steviol glycoside, an equivalent of 0-10 mg kg/ BW of stevioside. The steviol glycosides are currently in use as a sweetener in a number of industrial foods, such as soft drinks or fruit drinks, desserts, cold confectionery, sauces, delicacies, sweet corn, breads, biscuits, table-top sweetener. They replace sucrose, for example in ready-to-eat cereals, yoghurt, candies, soju, soy sauce and pudding.

Conclusion

From our study, it could be concluded that Stevia plants is a good nutritious source of carbohydrates, protein, crude fiber, minerals essential and non-essential amino acids which are vital for human nutrition and maintains a good health. Aqueous stevia extract showed very high significant correlation (P< 0.05) in improving HDL and reducing LDL, total cholesterol and triglyceride profile for hypercholesteremic albino rats. Powder stevia leaves had positive microbial properties against *S. typhimurium*. Nutritious (energy content, sweetness and hypolipidimic characteristics) and economic advantages of stevia recommended to be applied in food processing and appear its vital role in therapeutic nutrition especially with diabetic, heart disease and weight control patients.

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