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Influence of the harvesting area on the nutritional value, antioxidant and hypoglycemic properties of *Spirulina platensis* (Gom.) in diabetic rats (type I diabetic)

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Abstract

Background: The present investigation aimed to study the effect of the harvesting area on the nutritional value, antioxidant and antidiabetic properties of Spirulina (*Spirulina platensis*) from five zones in type 1 diabetic rats.

Methods: *Spirulina platensis* was collected in dried forms from five zones (Lake Bodou, Lake Kolodorum, Lake Lactir, Lake Touffou and the wadi of Rombou) and then subjected to hydroethanolic extraction (50-50). The nutritional value, the antioxidant potential total phenols and flavonoid contents of the extracts were evaluated. The extracts were subsequently administered by oral intubation to diabetic rats induced by intraperitoneal injection of streptozotocin (60 mg/kg). The fasting blood glucose was weekly taken during the three-week experimental period. Afterwards, blood was collected and the serum prepared for measurement of biochemical parameters.

Results: The results showed that the spirulina extracts from Touffou Lake (SP4) has the highest level of protein (71g/100g of dried matter (DM)), carbohydrates (23g/100g DM), fibres (1.5g/100g DM) and minerals. Spirulina extracts from Touffou Lake (SP4) and from Rambou wadi (SP5) have the highest antiradical and antioxidant activity. They also caused a significant reduction in glycemia, serum triglyceride, total cholesterol, LDL cholesterol, serum transaminases (ALAT and ASAT), creatinine, urea, and total protein levels. Activities of catalase and peroxidase in the tissus were significantly increased for the group receiving spirulina extract from Lake Touffou compared to other groups.

Conclusions: The nutritional value, the antioxidant and the hypoglycemic potentials of spirulina are influenced by the harvesting area.

Keywords: Antioxidant; Harvesting area, Diabetes, Nutritional Value, *Spirulina platensis*

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Background

Diabetes is a serious chronic disease, which occurs when the pancreas does not produce enough insulin or when the body does not properly use the insulin produced [1]. In 2015 WHO estimated that nearly 1.6 million deaths were directly related to diabetes and predicted that in 2030, diabetes would be the seventh cause of death in the world if nothing is done [2]. [The global prevalence of diabetes among adults over the age of 18 increased from 4.7% in 1980 to 8.5% in 2014 [2]. In Cameroon, the male prevalence of diabetes was estimated at 6.5% without distinction of the living environment in 2003 while this prevalence in women was 8.5% and 5% respectively in urban and rural areas at the same period [3]. The primary purpose of diabetes treatment is to maintain normal or near normal glucose levels, to achieve normal weight, to prevent and treat complications of diabetes [4]. The adverse effects of antidiabetics, their difficult administration and their high costs force researchers to turn on to news methods of treatment based on functional foods and phytotherapy.

Spirulina platensis (*Arthrospira platensis*), a cyanobacteria that was declared "best food source of the future" by the United Nations during the World Food Conference of 1974 [5] is used as a supplement food. It contains on average dry weight, nearly 70% of proteins, 15 to 25% of carbohydrates and 11% of lipids as well as vitamins, minerals (mainly trace elements), chlorophyll and phycobiliproteins. In Chad, this blue alga covers an area of 136.25 hectares, it is in the two departments of Kanem and Lake, located north and north-west of Lake Chad between the 14th and 17th parallel of atitude in the Northern Hemisphere [6]. The growth of spirulina is preferentially done in warm waters, alkaline and rich in nitrogen and phosphorus nutrients, it is generally observed in brackish water, as well as in saline lakes in the tropics and semi-tropics [7]. Previous studies on Spirulina showed that it is endowed with lipid-lowering, antidiabetic and antiobesity properties as well as reduction of the effects of heavy metals and drugs and protection against radiation [8]. *Spirulina platensis* (SP) can be obtained from different harvesting areas which can modify its composition and activity. Thus, this work was designed to assess the effect of the harvesting area on the nutritional value, the phytochemical content of Spirulina from 5 zones, in relation to their antioxidant and antidiabetic potential.

Methods

Plant material

Blue-coloured SP was purchased in dried form in Chad at the following area: Bodou Lake (SP1), Kolodorum Lake (SP2), Lactir Lake (SP3), Touffou Lake (SP4) and the wadi of Rambou (SP5) and transported to the Research Unit of Medicinal Plants Biochemistry, Food Science and Nutrition of the Biochemistry Department of the University of Dschang-Cameroon where they were sorted out for the elimination of waste before use.

Powders and aqueous extracts preparation

After collecting dried samples they were ground using a blender. Then the different powders were obtained as per harvesting area and kept in a desiccator. The extracts obtained (hydroethanolic extracts) were made using the method described by Iqbal and Bhanger [9]. Eight (8) g of powder was macerated in 110 mL of hydroethanolic solvent (50/50) and placed at room temperature for 48 h. The mixture was then filtered using the Whatman paper $N^0 1$ and the residues collected in 300 mL of solvent under the same conditions to increase the extraction yield. After filtration, the filtrate was placed in the oven at 45 °C until obtaining a constant weight.

Determination of chemical composition

The determination of lipids, proteins, fibers and carbohydrates content was performed by the methods AOAC [10], and the minerals content (calcium, magnesium, potassium, phosphorus, iron and sodium) by the method of Pauwels et al. [11].

Phytochemical Tests

Determination of polyphenol content using the folin-Ciocalteu reagent

The hydroethanolic extracts of the different SP extracts were analyzed by the determination of the total phenolic compound content using the Folin-Ciocalteu method as described by Dohou *et al.,* [12]. To 0.01 mL of a solution of concentration extract (2 mg/mL), we added 1.39 mL of distilled water and 0.2 mL of folin-Ciocalteu reagent. After 3 min of rest, 0.4 mL of sodium carbonate (Na2CO3, 20%) was added. The tubes were then agitated using a vortex and incubated for 20 min in a water bath at 40 °C and the absorbance was read against a blank at 760 nm using a spectrophotometer using a BioMate 6 UV–vis spectrophotometer (BIOMATE Thermo Scientific, Madison, WI, USA). The total phenolic content was determined using the standard curve (y = 0.022 x; r2 = 0.9945) obtained with Gallic acid. The contents were expressed as mg of Gallic Acid Equivalent/g of extract/powder.

Dosage of flavonoids

Aluminum trichloride method, as described by Padmaja et al. [13] was used to determine the total flavonoid content of the five extracts of *SP*. One hundred (100) µl of extract was mixed with 1.49 mL of distilled water before introducing 0.03 mL of 5% NaNO2. After 5 min, 0.03 mL of 10% AlCl3 was added and the mixture was allowed to rest. After 6 min, 0.2 mL of 1 M NaOH and 0.24 mL distilled water were respectively added, the mixture was vortexed and the absorbance was measured at 510 nm using a BioMate 6 UV–vis spectrophotometer (BIOMATE). The flavonoid content was determined using the standard curve (y = 0.1972 x; r2 = 0.9972) obtained with Catechin. The contents were expressed as mg CE/g of extract/suspension.

Measurement of antioxidant and anti-radical parameters

Determination of the percentage inhibition of the DPPH radical DPPH (2.2-diphenyl-1-Picrylhydrazyl)

Antioxidant activity of all samples of SP was measured using 2,2-diphenyl-1-picrylhdrazyl (DPPH) according to the method described by Mensor *et al.* [14]. Briefly, 100 µl of extract/powders' suspension was added to 900 µl of DPPH reagent (0.3 mM DPPH (2,2-Diphényl-1-picrylhydrazyl) solubilised in ethanol). After 30 min of incubation at room temperature, the absorbance was read at 517 nm against a blank; Butylhydroxytoluene (BHT) was used as standard.

FRAP (Ferric reducing antioxidant power)

The ferric reducing antioxidant power of different extracts as determined using the method of Padmaja et al. [13]. One mL of sample at different concentrations (200, 100, 50 and 25 µg / mL) was mixed with 2.5 mL of 0.2M phosphate buffer solution (ph = 7.4), and 2.5 mL of potassium ferricyanide solution K3Fe (CN) 6 at 1% (1g of K3Fe (CN) 6 in 100 mL of distilled water). The mixture was incubated in a water bath at 500 °C for 20 min then 2.5 mL of 10% trichloroacetic acid was added to stop the reaction followed by centrifugation at 300 rpm for 10min. Afterwards, an aliquot (2.5 mL) of supernatant was combined with 2.5 mL of distilled water and 0.5 mL of a 0.1% of freshly prepared ferric chloride solution was added and the absorbance was read against a blank at 700 nm.

Experimental animals and diets

Three-month Wistar rats weighing between 200 and 230 g were obtained from the Department Animal Centre and allowed to be accustomed to the new environment for 1 week. They were maintained in accordance with the guidelines of the OECD [14]. Afterwards, type I diabetes rats were obtained by intra-peritoneal injection of streptozotocin (60mg/kg weight) in rats [15]. Five days later rats with blood glucose level higher than 2g/l were considered as diabetic, and they were distributed in eighth groups of six rats each. A group of six non-diabetic rats was also used as a negative control. Then, the various hydroethanolic extracts of *SP* (1g/kg), glibenclanide (2.5 mg/kg) and distilled water were administered by gavage during twenty one days. All these experiments were carried out in accordance with the regulation and the ethical approval.

Estimation of biological parameters: Blood glucose levels, serum lipids, transaminases, total protein, urea, creatinine and antioxidant enzymes (peroxidase, catalase)

Blood glucose (mg/dl) was measured weekly from the tail tip after eight hours fasting, using a ACCU-Check glucometer (AccuChek Active, Roche Diagnostics, USA) throughout the treatment.The serum lipid profile was determined using colorimetric methods (TAYTEC kits). Standard protocols as described by Trinder [16] were used for the total cholesterol and HDL cholesterol while the LDL cholesterol was estimated using the formula established by Friedewald et al. [17].The colorimetric methods using the Chronolab kit were used to determine the serum levels of Aspartate animotransferase (ASAT) and Alanine animotransferase (ALAT), the standard protocol described by Reitman and Frankel [18]. The determination of plasma and serum creatinine levels was made using the method described in Bartels et al. [19] and urea by an association of the methods described by Fawcett and Scott [20].
Total protein determination was based on the Colorimetric method using the SGMitalia kit as described by Lowry et *al*., [21].

Statistical analysis

The data processing was done using the Microsoft Excel 2013 software. The results were expressed as mean ± standard deviation. The One Way Anova followed by Turkey Post hoc was used to highlight the significant differences between the averages at $p<0.05$ with the IBM SPSS 23 statistics software.

Results

Chemical Composition

Macronutrients: Table 1 below shows the protein, lipid, carbohydrate and fibre content of SP extracts from 5 different areas. We note here that there was a significant difference ($P < 0.05$) between the protein, carbohydrate and fibers content of the different SP

extracts. We can also observe that there was no significant difference (P>0.05) between the lipid content of extracts SP1, SP2 and SP4. *Mineral content:* Table 2 below shows the content of a few minerals present in SP from different areas, it appears that there is a significant difference (P<0.05) between the levels of calcium, magnesium and potassium from the different extracts. It can also be noted that *SP* from the harvesting area 4 (lake Touffou) had the highest calcium, phosphorus, magnesium, potassium, iron and sodium levels compared to others.

Table 1. macronutrients contents of SP Code extracts contents of macronutrients (g/100g)

Extracts	Contents in macronutrients (g/100g)			
	Proteins g)	Lipids (g)	Carbohydrate (g)	Fiber (g)
SP1	62,50±0,50^{b}	6,00±0,9^{a}	16,50±0,50^{b}	0,80±0,006^{c}
SP2	59,00±0,01^{a}	6,00±1,00^{a}	14,50±0,50^{a}	0,60±0,004^{a}
SP3	68,50±0,50^{c}	7,50±0,50^{b}	19,00±0,08^{c}	0,65±0,050^{b}
SP4	71,00±0,06^{e}	5,90±0,10^{a}	23,00±1,00^{d}	1,50±0,003^{d}
SP5	69,50±0,50^{d}	8,00±0,03^{b}	18,00±1,00^{c}	0,80±0,01^{c}

SP1: SP extract from Bodou Lake; SP2: SP extract from Kolodorum Lake; SP3 extract of SP from Lactir Lake; SP4 extract of SP from Touffou Lake; SP5 extract of SP from the wadi of Rambou. Values with different letters (a, b and c in the column) are significantly different at P<0.05

Table 2. Contents of a few minerals of SP

EXTRACTS	Mineral content (mg/100g)					
	Calcium	Phosphorus	Magnesium	Potassium	iron	Sodium
SP1	761,00±1,00^{a}	1041,00±1,00^{a}	512,25±0,25^{a}	1056,50±0,50^{a}	566,00±0,00^{a}	619,50±0,50^{a}
SP2	771,00±1,00^{e}	1040,60±0,40^{a}	513,50±0,50^{b}	1068,00±1,00^{b}	567,00±0,00^{b}	621,50±0,50^{b}
SP3	763,50±0,50^{b}	1043,00±0,00^{b}	514,25±0,25^{b}	1070,50±0,50^{c}	567,50±0,50^{bc}	620,25±0,25^{a}
SP4	768,50±0,50^{d}	1046,00±1,00^{c}	522,35±0,35^{d}	1075,00±1,00^{d}	568,50±0,50^{d}	622,50±0,00^{c}
SP5	766,00±0,00^{c}	1045,00±1,00^{c}	516,00±1,00^{c}	1071,00±1,00^{c}	568,00±0,00^{cd}	622,50±0,50^{c}

SP1: SP extract from Bodou Lake; SP2: SP extract from Kolodorum Lake; SP3 extract of SP from Lactir Lake; SP4 extract of SP from Touffou Lake; SP5 extract of SP from the wadi of Rambou. Values with different letters (a, b and c in the column) are significantly different at P<0.05

Antioxidant and anti-radical activity of the various SP extracts

Total phenolic Contents: Figure 1 below shows the total phenol contents of the different extracts of *SP* from the different harvesting areas. It appears that there was a significant difference (P < 0.05) between the phenol content of the extracts SP2, SP4, SP5 versus SP1 and SP3.

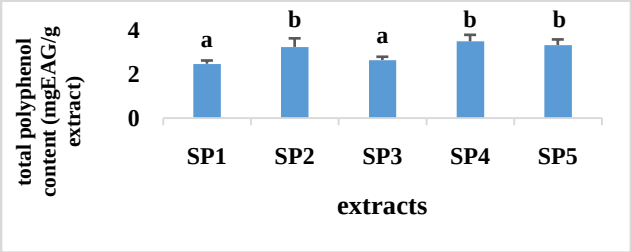

Figure 1. Total phenolic contents.
SP1: SP extract from Bodou Lake; SP2: SP extract from Kolodorum Lake; SP3 extract of SP from Lactir Lake; SP4 extract of SP from Touffou Lake; SP5 SP extract from the Wadi of Rambou. Values with different letters (a, b and c on the diagrams) are significantly different at P<0.05

Flavonoid contents: Figure 2 shows the flavonoid contents of the different extracts of *SP*. In this figure, it appears that there was no significant difference (P < 0.05) between the contents of the SP extracts SP4 and SP5. The same observation was made between the SP2 and SP3 extracts. We can also noted that the lowest flavonoid content was obtained for SP1 extract.

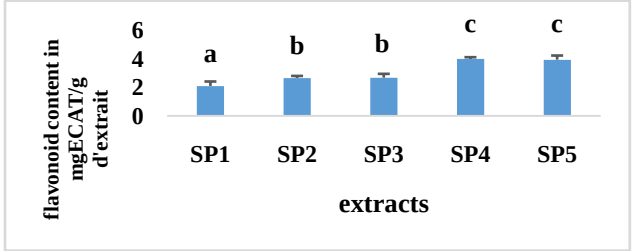

Figure 2. Flavonoid contents of the different extracts
SP1: Extract of SP from Bodou Lake; SP2: SP extract from Kolodorum Lake; SP3 extract of SP from Lactir Lake; SP4 extract of SP from Touffou Lake; SP5 extract of SP from the wadi of Rambou. Values with different letters (a, b and c on the diagrams) are significantly different at P<0.05

DPPH Scavenging Ability: The percentage of inhibition of the DDPH radical as a function of the concentration (µg/mL) of the extracts is described in Figure 3 below. It should be noted that all *SP* extracts exhibited scavenging activity but at varying and very low proportions in comparison with BHT. The extract with the highest percentage was obtained from Region 4 (Touffou Lake) with a value of 40.15 ± 0.59 µg/mL at the concentration of 200 µg/mL.

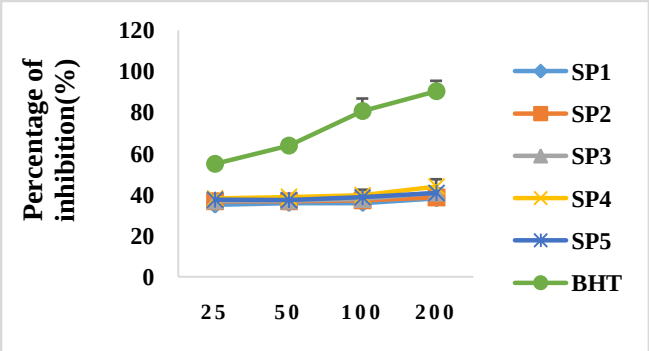

Figure 3. Evolution of the anti-radical activity of the different extracts of SP.
SP1: SP extract from Bodou Lake; SP2: SP extract from Kolodorum Lake; SP3 extract of SP from Lactir Lake; SP4 extract of SP from Touffou Lake; SP5 extract of SP from the wadi of Rambou.

The Figure 4 below shows the IC_{50} of our different SP extracts. It is seen that SP extracts have a relatively high IC_{50} that ranges from 179.02 ± 1.73 to 191.43 ± 2.05 µl/mL, compared with BHT. It is also noted that there are no significant (P>0.05) differences between IC_{50} of SP extracts from Lactir lake (SP3), Touffou lake (SP4), and Rambou wadi (SP5).

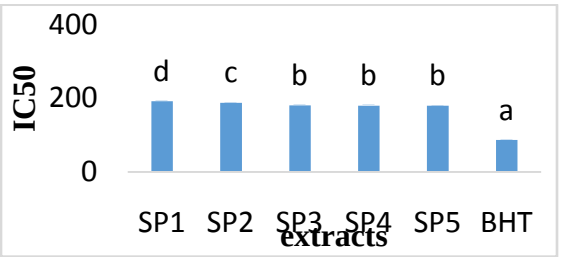

Figure 4. IC_{50} of the different extracts compare to that of BHT
SP1: Extract of SP from Lake Bodou; SP2: SP extract from Kolodorum Lake; SP3 extract of SP from Lactir Lake; SP4 extract of SP from Touffou Lake; SP5 extract of SP from the wadi of Rambou. Values with different letters (a, b and c on the diagrams) are significantly different at P<0.05

Total reduction of Fe3 + (FRAP): In Figure 5 it appears that the reducing power of vitamin C is significantly (P < 0.05) higher than that of the individual extracts. We also noted that all SP extracts had the ability to reduce iron regardless of the harvesting area, but with very little variable values. The Extract from Zone 4 (Touffou Lake) showed the highest iron reducing power (0.22 ± 0.01) at the concentration of 200 µg/mL.

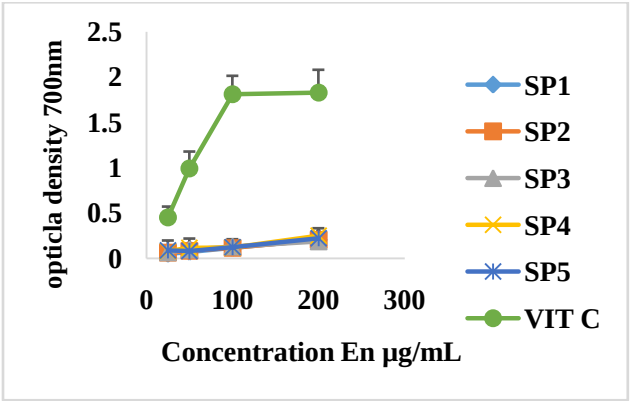

Figure 5. Reducing power of the different extracts of SP compared to that of vitamin C.
SP1: SP extract from Bodou Lake; SP2: Extract of SP from Kolodorum Lake; SP3 extract of SP from Lactir Lake; SP4 extract of SP from Touffou Lake; SP5 extract of SP from the wadi of Rambou ; vit c vitamine C.

Antidiabetic Tests

Blood glucose level during treatment: Figure 6 shows the results of fasting blood glucose changes in different groups throughout the experimental period. It is noted that after 21 days of treatment, a reduction in blood glucose was observed in all groups except those with untreated diabetics (TDNT) (where blood glucose levels increased between the onset and end of treatment) and the healthy control group (TS). It was also observed that the group receiving SP extract from Touffou lake (SP4) decreased compared with the other groups.

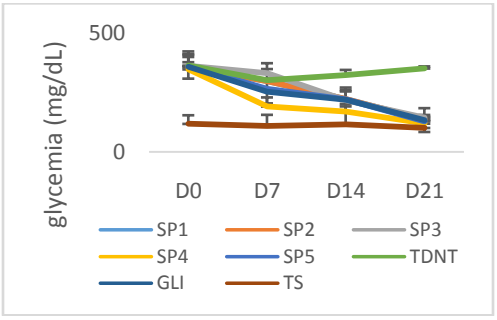

Figure 6. Changes in blood glucose levels during treatment. *SP1: SP extract from Bodou Lake; SP2: Extract of SP from Kolodorum Lake; SP3 extract of SP from Lactir Lake; SP4 extract of SP from Touffou Lake; SP5 SP extract from the Wadi of Rambou TDNT (untreated diabetic control) GLI (group receiving glibenclamides), TS (healthy control), Gly(Glycemia).*

Effect of Hydroethanol extract on serum lipid profile: Figure 7 (A-D) shows serum levels of biochemical parameters for animals following treatment with hyrdoethanolique extract of different SP. Figure 7A indicates that there is a significant (P<0.05) difference in serum triglyceride levels between controls and groups following treatment. As a result, serum triglyceride levels in untreated diabetic rats (TDNT) are significant (P<0.05), compared with those of non-diabetic rats (TS) and those treated with different SP extracts. Figure 7B indicates that no significant (P<0.05) difference exists in serum total cholesterol levels between healthy controls and the groups that have followed the treatment with SP extracts. Figure 7C shows serum levels of HDL cholesterol for animals following treatment with different SP extracts. It appears that in the groups treated with the different SP extracts, there is a significant (P<0.05) increase of the plasma concentration of HDL. Figure 7D shows the level of LDL in animals, resulting from this figure that there is a significant difference between groups receiving SP extracts and those with untreated diabetics. It is also noted that there is no significant difference between SP extracts from areas 4 (Touffou lake) and 2 (Kolodorum lake).

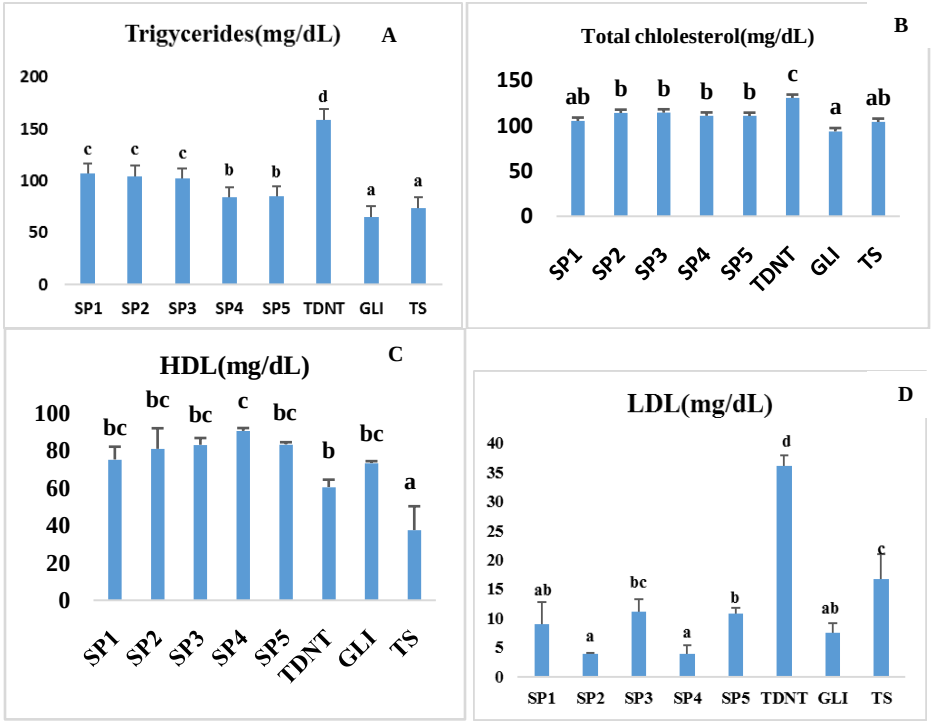

Figure 7 (A, B, C and D). Serum triglyceride levels, total cholesterol, HDL cholesterol, LDL cholesterol
SP1: SP extract from Bodou Lake; SP2: SP extract from Kolodorum Lake; SP3 extract of SP from Lactir Lake; SP4 extract of SP from Touffou Lake; SP5 SP extract from the Wadi of Rambou TDNT (untreated diabetic control) GLI (group receiving glibenclamides), TS (healthy control). Values with different letters (a, b and c on the diagrams) are significantly different at P<0.05

Effect of Hydroethanolic extract on markers of hepatic and renal damage: Figure 8 (A-D) shows serum levels of biochemical parameters on hepatic damage (alanine aminotransferase, aspartate aminotransferase) and renal (creatinine and urea) Figures 8A and 8B show serum transaminases levels. It is noted that untreated diabetic rats have serum levels of transaminases (ALAT and ASAT) higher than those of controls. It can also be noted that there is no significant difference between the groups receiving the SP4 and SP5 extracts. Figures 8C and 8D show the levels of creatinine and plasma urea. On these figures, untreated diabetic rats exhibited significantly (P<0.05) elevated serum creatinine levels compared with those of treated diabetic controls.

In Figure 9, the serum protein concentration in untreated diabetic rats was significantly (P<0.05) higher compared to those receiving the extracts. We also noted that there was no significant difference between the different SP's extracts.

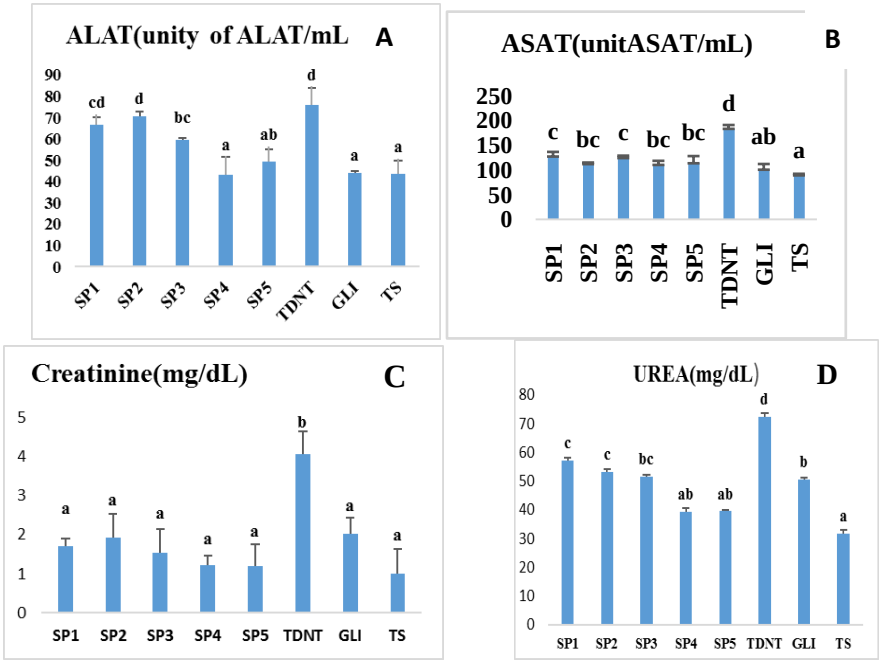

Figure 8(A,B,C,D). Serum levels of ASAT, ALAT, urea and creatinine levels.
SP1: Extract of SP Provenantbdu Lake Bodou; SP2: SP extract from Kolodorum Lake; SP3 extract of SP from Lactir Lake; SP4 extract of SP from Touffou Lake; SP5 SP extract from the Wadi of Rambou TDNT (untreated diabetic control) GLI (group receiving glibenclamides), TS (healthy control). Values with different letters (a, b and c on the diagrams) are significantly different at P<0.05

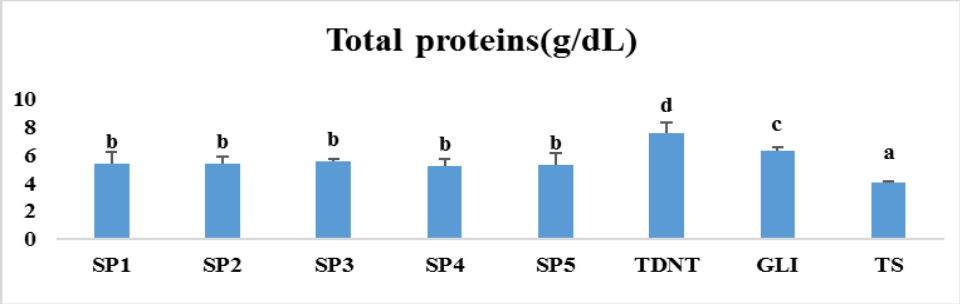

Figure 9. Serum protein levels.
SP1: SP extract from Bodou Lake; SP2: SP extract from Kolodorum Lake; SP3 extract of SP from Lactir Lake; SP4 extract of SP from Touffou Lake; SP5 SP extract from the Wadi of Rambou TDNT (untreated diabetic control) GLI (group receiving glibenclamides), TS (healthy control). Values with different letters (a, b and c on the diagrams) are significantly different at P<0.05

Discussion

The protein content found in our study varies from 62.5 to 71% depending on the harvest area, and these values remain within the range determined by Belay [22], who proved that the protein content of SP varies from 60% to 74%. In terms of carbohydrate content, lipids and fibres the results of this study are similar to those of the Centre for the study and enhancement of algae [CEVA] [23] which found that SP contains 7% lipid, 2% fibers and 10% carbohydrates. For phosphorus, potassium and sodium the values are similar to those of CEVA in 2015, which found values of 916mg, 1000mg, and 641mg, respectively, for phosphorus, potassium, and sodium. With regard to the values of calcium, magnesium and iron they belong to the interval [24]. These differences in content in our SP samples can be explained by the period that the SP were harvested, the strain, the culture medium and the drying technique and the method of preservation; in fact, temperatures between 28 and 32^{0}C, and a pH of 11, are favorable conditions for optimum growth [25]. However, changes in nutrient content could be explained by the harvesting technic that was done in a traditional way. In fact, the presence of sand (more than 30%), living worms, plant debris, animals and insects can also adversely affect the nutritional quality

of SP according to the Chadian Institute for Agronomic Research for Development [26].

The phenol contents found in our extracts are between 2.46 ± 0.14 and 3,48 ± 0.29 of gallic acid equivalent/g extract. The analysis of this figure shows that the highest total phenolic content in the SP extract was obtained from zone 4 (Lake Touffou) (3,48 ± 0.29 mg EAG/g extract), followed by extract from Zone 5 (Wadi of Rambou) (3,31 ± 0.25 mg EAG/g extract). These contents belong to the range of Collado et al., [27], who found the content in total phenolic compounds of SP between 2.4 and 5.0 mg Eq AG/g extract. With respect to flavonoids, it is clear that the SP extracts from Touffou Lake (SP4) and Rambou Wadi (SP5) were significantly higher than others. These results are within the concentration range given by Hanaa et al., [28] (1.32 to 5.12 mg ECAT/g extract). The difference between phenol and flavonoid contents of our extracts may be related to the drying temperature, because Colla et *al.*, (2006) [29] showed that drying temperature is important for the production of biomass, protein, lipids and phenolic compounds of *SP*. A temperature of 35 °c and a pH of 11 have a negative effect on biomass production and a positive effect on the production of proteins, lipids and phenolic compounds [25]. In addition, environmental factors such as sun exposure, soil type, water composition, and precipitation have an effect on the phenol content of plants [30]. Indeed the low temperature can increase the production of phenols, improving the synthesis of phenylalanine ammonia lyase (PAL) in plants, while exposure to high altitude and sunlight with high UV radiation positively influence the phenolic compounds synthesis [31].

All SP extracts had the ability to trap the DDPH radical with different percentages of inhibition. These results are similar to those of Anbarasan [32], who found an activity of 27.88 ± 1.21 at a concentration of 100 µg/mL. All IC_{50} were very high, and varies from 179.02 ± 1.73 to 191.43 ± 2.05 µg/mL compared to BHT. These results are close to those of Yahiaoui [33] who found an IC_{50} of 182.3 µg/mL. The antiradical activity of the extracts could be explained by the presence of polyphenolic compounds (anthocyanins, flavonoids...), as confirmed by the phytochemical test. Phenolic compounds and nitrogen compounds are potential antioxidants because they possess an ability to neutralize free radicals and active oxygenated species such as hydroxide radicals or singlet oxygen [34, 35]. The extracts SP4 (Lake Touffou) and SP5 (wadi of Rombou) with higher percentages. The difference between the antiradical activities of our SP extracts may be explained by their levels of phenolic compounds and the characteristics of the harvesting area. Indeed the work of Disna et al., [36] on the comparison of phenolic compound content, and antioxidant activities of millet varieties grown at different locations in Sri Lanka showed that phenolic content as well as antioxidants activities of soluble and bonded mil phenolic extracts were affected by the variety and harvesting area.

The ability of our different extracts to reduce iron would be due to the presence of phenolic compounds. Thus Verdan et al., [37] reported that the antioxidant effect of flavonoids is due in part to their ability to fix different metals (Fe^{2+}, Fe^{3+}, Cu^{2+} and Zn^{2+}) on several sites. So the more an extract is rich in phenolic compounds, the more it will have the ability to fix the metals.

The differences between antioxidant and antiradical values of SP extracts can be related to the harvesting conditions of *SP*, the composition and concentration of the culture medium ($NaNO_3$, phenylalanine,...), pH, temperature, pressure, CO_2 content, light intensity (photosynthesis), seasons, geographical location, climatic and environmental conditions (heavy metals and ionization). Indeed, the work of Xu-Dan et *al.*, [38] has shown that environmental factors such as temperature and sunlight exposure can be important factors affecting some antioxidant and anti-radical properties of Buckwheat flour.

Several studies have shown that diabetes causes biochemical changes (disruption of sugar, protein and lipid metabolism) and also an increase of oxidative stress at the origin of microangiopathic and macroangiopathic diabetes complications. In this study, rats made diabetic (type I diabetes) by injecting a dose of streptozotocin showed a significant increase in blood glucose levels compared with healthy rats. This result is in agreement with those of Hamadi [39] who reported a significant increase in the rate of glucose in the blood. Blood glucose reduction in hyperglycemia animals may be due to the presence of hypoglycemic substances that act by stimulating the release of insulin [40]. Indeed, the results of lower blood glucose levels in the animals treated with SP extracts are consistent with those of Gargouri et al., [41] who showed that SP is effective in inhibiting hyperglycemia, and the oxidative stress induced by diabetes. However, this decrease in blood glucose levels and the variation in blood glucose levels in groups receiving different SP extracts can be justified by the chemical composition of our different SP extracts because it contains a large quantity of good quality protein that after digestion, will release peptides and polysaccharides that can stimulate the secretion of insulin and consequently reduce blood glucose levels [42]. This can explain the differences in blood glucose levels between the different groups receiving SP extracts. Moreover, the decrease of blood glucose level is due to the presence of flavonoids in SP [43], as they may act by inhibiting the alcalose reductase. So, the more an extract is rich in flavonoids, the more it will have the ability to

reduce blood glucose effectively and this can explain the very significant drop in blood glucose in the group receiving the extract SP4 (lake Touffou). Lipids play an important role in the pathogenesis of diabetes mellitus. The level of serum lipids is generally high in diabetes and such elevation represents a risk factor for coronary and heart diseases [44]. The increase in triglyceride levels in untreated diabetic rats compared to treated rats could be attributed to vascular complications that causes lipid metabolism disorders. Thus, Daisy et al., [45] suggest that a high concentration of serum lipids observed in diabetic subjects is mainly due to increased mobilization of fatty acids from adipose tissues since insulin inhibits lipase hormone because insulin activates the enzyme lipoprotein lipase. The results of the decrease in triglyceride levels in animals receiving SP is in agreement with those Ngo-Matip et *al*., and Rodriguez-Hernândez et al., [46, 47] who have shown that *SP* lowers the triglyceride levels. Thus, extracts from Zone 4 and 5 may have a very large capacity to stimulate hormone lipase, which will result in a significant decrease in triglyceride levels compared to other extracts. The decrease in cholesterol levels in the rats treated with SP extracts can be explained by the presence of fiber and phenolic compounds in SP which confers its hypolipidemic effect. Indeed, the Phycocyanin present in SP increases the reabsorption of bile acids at the ileum level, carotenoids, γ-linolenic acid, fibers and sterols reduce the jejunal and ileal absorption of cholesterol and inhibit the expression of the hepatic HMG-CoARéducatse (3-Hydroxy 3-methylglutaryl CoA reductase), the enzyme responsible for the biosynthesis of cholesterol. The increase in cholesterol levels in untreated diabetic rats may be due to the absence of insulin, caused by the destruction of the β cells of the pancreas following injection of streptozotocin. Indeed Betterridge et al., [48] report that insulin deficiency may be responsible for hyperglycemia because insulin Inhibitory action on the rate of HMG-COA (3-hydroxy-3-methyl-glutaryl coenzyme A reductase, key enzyme in cholesterol biosynthesis. These results are consistent with those of Bashandy et al., [49] showed that SP has an effect on lowering cholesterol levels. The difference between the cholesterol levels of SP extracts can be explained by the fact that the SP extracts from different harvesting areas do not have the same chemical composition. Differences in the fibre and anti-oxidant content of the different hydroethanolics extracts of *SP* could explain the HDL levels in the different groups receiving SP. Thus, the high concentration of these various substances could explain the ability of the extracts to increase the serum level of HDL cholesterol. This work is consistent with those of Chao et al. [50], which showed that bioactive compounds, including fibers in SP, have a hypolipidemic effect. The decrease in the rate of LDL in animals receiving SP could be explained by the presence (in higher quantities) in SP extracts of hypolipidemic compounds, including raw fibers and phenolics. The decrease in LDL levels is consistent with those of Zeweil et al., [51], which showed that a decrease in LDL levels in chickens supplemented with heat-stressed SP.

The decrease in serum transaminases levels observed in this work compared with control could reflect the hepatoprotective effect of the various *SP* extracts in rats. It is also noted that the group receiving the Touffou lake SP extract (SP4) has a relatively low ALAT ratio as compared to the others. The rate of transaminase in normal subjects is equal to that of subjects treated with glibenclamide and due to the fact that normal subjects have a rate of transaminases (ALAT and ASAT) elevated in the cytoplasm. In case of hepatic dysfunction or lesion, there is displacement of these enzymes to the extracellular medium, leading to an increased levels of these parameters in the serum [52]. Untreated diabetic rats had serum ALAT and ASAT levels significantly ($p < 0.05$) higher than those of non-diabetic rats. An increase in the activity of these enzymes reflects damage to the active liver and hepatocellular inflammatory disorders [53]. Indeed, the lipid peroxidation of the cellular membrane and the hepatic endoplasmic reticulum following oxidative stress, which is induced by diabetes, makes the cell membrane porous and leads to the release of ALAT and ASAT in the blood. Equally, the decrease in serum protein levels observed in this work confirms the hepatoprotective effect of the extracts, which limits the degradation of hepatocytes by decreasing the amount of serum transaminase and other enzymes that must be released into the bloodstream, as an increase in serum proteins would reflect a cellular lesion [54].

The increase in creatinine and urea in untreated diabetic rats could be related to renal dysfunction in untreated diabetic rats, which is due to the nephrotoxicity of streptozotoccin [55]. Indeed, the accelerated degradation of proteins or the alteration of nitrogen homeostasis which leads to the increase of the hepatic elimination of nitrogen in the form of urea, can also explain the high rate of urea [56]. Therefore, the treatment of rats by glibenclamide and the different SP extracts could cause a decrease in urea and creatinine in the blood and therefore help protect the kidneys from streptozotocin-related toxicity. Our results are consistent with those of Palsamy and Subramanian [57], who noted a similar effect, with Resveratrol as a flavonoid. Urea being the final product of protein catabolism, one might also think that it is the strong degradation of proteins that is responsible for the presence in high quantity of urea in the serum. Thus, the SP4 and SP5 extracts have the strong ability to reduce the damage caused

by diabetes at the kidney level. This could be justified by the high content of bioactive compounds (fibers and phenols).

Conclusions

In conclusion, the SP harvesting area has an influence on its chemical composition, antioxidants and antidiabetic properties in type 1 diabetic rats. Thus, *Spirulina platensis* from lake Touffou and the wadi of Rambou have a high content of macronutrients including proteins, carbohydrates, fibers and also some minerals like iron, calcium, phosphorus and magnesium which are endowed with the best *in vitro* and *in vivo* antioxidant activities. It is also noted that the various SP extracts have resulted in a reduction in blood glucose levels, serum total cholesterol, triglycerides, LDL cholesterol, transaminases, urea, creatinine and an increase in the rate of catalase and rat peroxidases after 21 days of treatment.

Abbreviations

ALAT : Alanine animotransferase ; ASAT :Aspartate animotransferase ; AOAC : Association of Official Analytical Chemists; HMG-COA: 3-hydroxy-3-methyl-glutaryl coenzyme A reductase

Authors' Contribution

DK, JEO is involved in designing the experiment. PFD, BBJ provided the spirulina . PFD, BBJ, CYW and CA performed the experimental work. BGKA, APNK, PTN, FAT were involved in statistical analysis. PFD and DK wrote the first and final draft. All the authors read and approved the final draft.

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Conflict of interest

The authors declare no conflict of interest

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