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Antioxidant and Anti-Inflammatory Activity of *Arthrospira platensis* Var. Lake Chad

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Original Research Article

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Abstract: The objective of this study is to evaluate the antioxidant and anti-inflammatory activity of the aqueous extract of *Arthrospira platensis* var. Lake Chad, also call spirulina. A phytochemical screening of the aqueous extract was done for the characterization of the secondary metabolites. The microbiological quality was estimated and essays of acute toxicity were made. The anti-inflammatory activity was determined by the method of the oedema led by carrageenan; total phenols were determined and the antioxidizing activity was estimated by the method of trapping of the radicals free of the DPPH. The phytochemical test showed the presence of tannins, flavonoids, sterols, saponosides and alkaloids. Subsequently, during the microbiological quality control, no presence of total coliforms, fecal coliforms, molds and salmonella was observed. As well as during the test of acute toxicity, no behavior disorder, no death was observed, even at the highest orally dose tested of 5000 mg/kg (DL> 5000 mg/kg). For the anti-inflammatory effect, the doses of 250 and 500 mg / kg of aqueous *Arthrospira platensis* extract were administered to the rats. After injection of carrageenan under the plantar aponeurosis of the right posterior paw of the rat, paw edema was measured at first at 30 minutes, then every hour during 5 hours, using a caliper. The results showed a significant dose-dependent reduction in paw diameter ($p < 0.05$), with inhibition percentage of 20.45% and 31.92%, respectively. These effects are comparable to that of dexamethasone which is a reference anti-inflammatory drug. The dosage of total phenols of aqueous extract revealed a content in total phenols of 0.647 mg EAA/g ES. The antioxidant activity of the aqueous extract showed a trapping effect on the DPPH radical with an IC_{50} = 0.056 mg / ml. These values are comparable to those of the reference antioxidant (ascorbic acid). The obtained results demonstrated that *Arthrospira platensis* var. Lake Chad aqueous extract possesses an anti-inflammatory and antioxidizing activity. These properties can be used in the prevention of the inflammatory diseases and that relating to oxidative stress. The results obtained showed that the aqueous extract of *Arthrospira platensis* var. Lake Chad has an anti-inflammatory and antioxidant activity. These properties can be used in the prevention of the inflammatory diseases and that relating to oxidative stress.
Keywords: Anti-inflammatory, antioxidant, *Arthrospira platensis*, Lake Chad.

INTRODUCTION

Substances of natural origin occupy an important place in the discovery of new medicine. We consider that about 50 % of the therapeutic agents used actually result from natural sources (plants, mushrooms, animals, seaweeds, etc.). We also consider that less than 10 % of the botanical species was studied for their biological activities [1]. According to the World Health Organization (WHO), approximately 65-80 % of the world population in developing countries, because of the poverty and because of the lack of access to the modern medicine, depend essentially on the traditional medicine for their health care primary sector. And in spite of the remarkable progress in organic chemistry of synthesis of the twentieth century, more than 25 % of medicine prescribed in industrialized countries find directly or indirectly their origin from the botanical species [2].

The inflammation and the oxidative stress are involved in a large number of human pathologies which have an enormous impact on the health of the populations. The inflammation is a way of natural defense of the superior species against any outside aggression (infection, wound mechanical aggression, etc.). It essential role is the elimination of the

pathogenic agent and the repair of the hurt tissue [3]. Sometimes the inflammation can be fatal because of the aggressiveness of the pathogenic agent, its persistence and the localization of the inflammation [4], where from the use in modern medicine of treatment with non steroïdian and steroïdian anti-inflammatory drugs. So, the oxidative stress, which is an imbalance of the oxidizing-antioxidant balance in favour of oxidizers, develops when the free radicals, oxidizing molecules, are more quickly produced than they cannot be neutralized by the body [5]. Recent works showed that substances of vegetable origin containing phenolic compounds generally, and flavonoids in particular, play an important role in the human health, because of their diverse pharmacological properties [6,7].

Arthrospira platensis is microscopic blue seaweed, pluricellular, which grows naturally in tanks of alkaline water with high salt content in the subtropical and tropical zones, including America, Mexico, Asia and Central Africa. Among the large number of species of Arthrospira, three sorts species of spiruline are more intensely studied, because they are edible with a big nutritional value and a potential therapeutic interest, those species are: Arthrospira maxima, Arthrospira fusiformis and Arthrospira platensis which is the object of our study [8].

The aim of this study is to estimate the antioxidant and anti-inflammatory activities of aqueous extract of Arthrospira platensis var. Lake Chad, and its interest lives in the fact that anti-inflammatory drugs of synthesis have a cost and their consumption can lead to unwanted effects for the body. The highlighting of the anti-inflammatory and\or antioxidant properties, individual or coupled of the aqueous extract of Arthrospira platensis var. Lake Chad, which is accessible seaweed could be an alternative in the prevention of the arisen of the inflammatory pathologies and that caused by the oxidative stress.

MATERIALS AND METHODS

Material

Plant material

The plant material is the dried blue-green seaweed: Arthrospira platensis, produced by the SODELAC supplier of spiruline in the CHAD, harvested in November, 2016 on the Lake Bol (Lake Chad). The sample was identified at the laboratory of bacteriology of the General Hospital of Douala.

Animal material

Animals used for the realization of the pharmacological tests were females rats (Rattus norvegicus) of Wistar origin, weighing between 150 and 210 g, came from the pet shop of the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala. They were fed by water and by food constituted of: flour of corn, soya flour, cake of palm-kernel, flour of bone, powder of fish and of concentrated by vitamins. They were maintained in a temperature of 25°C, with sufficient aeration and a natural bright cycle.

Preparation of the plant sample

The plant sample for the dried state underwent a series of macerations to obtain a dry extract. 200 g of powder of Arthrospira platensis were wallowed in 4 l of water distilled at room temperature, under agitation during 24 hours, this operation was repeated twice. The mixture was filtered then with paper Whatman number 1. The obtained filtrate was concentrated in the steam room WIESHEU 7151 (45-50 ° C). The yield on extraction (R) was calculated with the formula:

R = (Mass of the obtained dry extract / mass of the expected powder) × 100

Phytochemical study

The phytochemical examination was made to identify the main families of secondary metabolites.

Test of tannins: in a test tube dissolve the aqueous extract in 2 ml of methanol, add 3 drops of iron chloride (FeCl3) to 2 %. The appearance after a few minutes of a blue-black colour is characteristic for gallic tannins, and blackish colour characteristic of catechic tannins [9].

Test of coumarines: in a test tube dissolve the aqueous extract in 2 ml of ethanol, add 3 ml of sodium hydroxide 10 %. After agitation of the extract, the appearance of a yellow tint indicates the presence of coumarins [9].

Test of anthocyanins: In a test tube dissolve the aqueous extract in 5 ml of methanol, add some drops of sulphuric acid 10 %, after agitation, add to the mixture some drops of ammonium hydroxide 10 %. The presence of anthocyanins is asserted by a blue-purple colour in basic environment [9].

Test of anthraquinones: in a test tube dissolve the aqueous extract in 5 ml of distilled water, add 5 ml of ammonium hydroxide 10 %. After agitation for a few minutes, the appearance of a red ring indicates the presence of anthraquinones [9].

Test of saponosides: in a test tube dissolve the aqueous extract in 10 ml of water distilled during 2 mn, then shake strongly. A persistent foam after 15 mn, confirms the presence of saponosides [9].

Test of flavonoids: in a test tube dissolve the aqueous extract in 5 ml of methanol, then add some drops of hydrochloric acid concentrated (HCl) and 3 shavings of

magnesium. The appearance of a pink-orangy or purplish colour, is characteristic of flavonoids [9].

Test of terpenes: in a test tube dissolve the aqueous extract in 5 ml of chloroform, add to the solubilized extract 5 ml acetic anhydride, then 2,5 ml of concentrated sulphuric acid. The appearance in the interphase, of a purple tint or purple, turning to the blue then to the green, characterize the presence of sterol or of steroids, and the appearance of a red-brick colour, turning to the purple characterize the presence of tri-terpene [9].

Test of alkaloids: to obtain the solution of Dragendorff, 0,85 g of nitrate of basic bismuth were dissolved in 10 ml of acetic acid and 40 ml of water (solution A), then were dissolved 8 g of Iodide of potassium in 20 ml of water (solution B), the solutions A and B were then mixed in equal volume.

The aqueous extract was then dissolved in 6 ml of methanol, and after agitation, 1 or 2 drops of Dragendorff reagents were added. The presence of an orangy colour is characteristic of the presence of alkaloids [9].

Test of reducing sugars: to obtain the Liqueur of Fehling, 45 g of copper sulphate was add in 100 ml of distilled water (aqueous solution A), then 200 g of salt of tartrate of sodium and 150 g of hydroxide of soda were dissolved in 100 ml of distilled water (aqueous solution B); the solutions A and B were mixed in equal parts to obtain the Liqueur of Fehling, which can be preserved for approximately 18 minutes. The aqueous extract was dissolved in 5 ml of distilled water, to whom was added 2 ml of solution Fehling (A+B); after boiling for a few minutes, the appearance of red precipitate indicates the presence of reducing sugars [9].

Pharmacological study

Microbiological quality control: the test of microbiological quality control of extracts was made according to the standards established by the European Pharmacopoeia [10].

Preparation of samples

Volume taking and suspension mother or first dilution:
- In a sterile flask, weigh 1 g of the aqueous extract on a precision balance;

- Add 9 ml of BPW (buffered peptone water) (leave the BPW until a temperature close to the ambient temperature before its use) and homogenize.

Decimal dilution

With a sterile pipette, decant 1 ml of the suspension mother in a tube of 9 ml of sterile BPW;

- Don't introduce the pipette into the suspension mother of more than 1 cm and avoid any contact between the pipette containing the inoculum and the sterile diluent;

- Mix carefully the test sample and the BPW to obtain the dilution in 10-2. Repeat if needed these operations on the dilution 10-2 and the following decimal dilutions by using in every dilution a new sterile pipette to obtain the dilutions 10-3, 10-4, 10-5, 10-6, 10-7.

The following stages must be made at the latest 45 mn after the preparation of the initial suspension.

Preparation of samples for the microbiological analysis

Enumeration of the total coliforms (TC) and the fecal coliforms (FC):

The principle is the one of the in-depth sowing on Mc Conkey agar

Sowing:
- in a Petri dish the Mc Conkey agar was poured and with a sterile pipette, 1 ml of the initial suspension were transferred in.

- The operation was repeated with 6 other boxes of agar by using the various successive dilutions of 10-2 to 10-7, having homogenized to allow the incorporation of the product in the environment on all the Petri dishes;

- A second layer of the same agar was added and the Petri dishes was left to solidify.

Incubation: 37 °C ± 1 °C during 24 ± 2 hours.

Interpretation: after incubation the boxes were examined to look for the presence of typical colonies of the total coliforms and faecal coliforms (red round colonies, diameters of colonies = 0,5 mm)

Enumeration of yeasts and total molds (TYM)
The principle is an in-depth sowing on Sabouraud agar.

Sowing
- in a Petri dish, the Sabouraud agar was poured and with a sterile pipette, 1 ml of the initial suspension was transferred in

- The operation was repeated with 6 other boxes of molded by using the various successive dilutions of 10-2 until 10-7, having homogenized to allow the incorporation of the product on all the Petri dishes;

- A second layer of the same agar was added the Petri dishes was left to solidify.

Incubation: 25 ° C ± 1 ° C during 72 am ± 2 am. Interpretation: after incubation, the boxes of molded was examined to look for the presence of typical colonies and their aspect (white colonies).

Expression of the results for enumeration of the TC, FC, TYM

Case 1: number of characteristic colonies between 10 and 150

$$Ne = \frac{\Sigma C}{v((n1+0,1\times n2))\times d}$$

Case 2: Number of characteristic colonies lower than 10 but upper to 4

$$Ne = \frac{\Sigma C}{v\times n1\times d}$$

Case 3: number of colony uncountable with the first dilutions and only the last one of which is held

$$Ne = \frac{\Sigma C}{v\times n1\times d}$$

Case 4: number of colonies from the first to the last dilution uncountable, thus upper to 150

N= 150 × 10*x* characteristic colonies

Case 1	**Case 2**	**Case 3**
N = Number of UFC by gram or by milliliter of initial product C = Sum of the colonies counted on two (2) held boxes, for two successive dilutions and a box of which at least, contains at least 10 colonies V = Volume of the inoculum applied to every box n1 = number of box considered in the first held dilution n2 = number of box considered in the second reserved dilution d = Factor of the first held dilution	Ne = number of UFC by gram or by ml of initial product C = Sum of the colonies counted in the first held dilution V = Volume of the inoculum applied to every box n1 = number of box considered in the first reserved dilution d = factor of the first reserved dilution	N = Number of UFC by gram or by ml of initial product C = Sum of the colonies counted in the last dilution V = Volume of the inoculum applied to every box n1 = number of box considered in the last reserved dilution d = Factor of the last reserved dilution

Search for Salmonella

Non selective pre-enrichment:

- Sowing in the BPW;

- incubation in 37 ° C during 18h ± 2 h, the culture A is obtained

Selective enrichment:

- 0,1 ml of the culture A obtained was transferred, in a tube containing 10 ml of moondweller's Broth;

- the circles sowed were incubated in 37 ° C during 18 to 24 h, the culture B were obtained;

- in the various Petri dishes the Hektoen agar was poured, then let to solidify and finally the Petri dishes were put in a steam room in 37 ° C.

Isolation and identification

- from the culture B obtained in moondweller's Broth after incubation, with a handle the surface of the Petri dishes containing the Hektoen agar was sowed, the boxes were turned upside down and put in a steam room in 37 ° C;

- after 12 h ± 3 h of incubation, the various Petri dishes were examined to look for the presence of typical colonies of Salmonella, as well as the atypical colonies susceptible to be Salmonella.

Acute toxicity study

The acute toxicity test was conducted following the protocol of OECD Test Guideline 423 [11]. Three female rats were used for the determination of the DL50, aged from 8 to 12 weeks, weighing between 150 - 180 g. They were randomly selected and fasted for 12 hours before the test, but receiving water at will. After this fast, female rats were weighed and the trial substance was orally administered to them through an oro-gastric tube according to the following distribution:

- The batch 1: distilled water 2 ml / 100g of body weight

- The batch 2: extract at the dose of 2000 mg / kg of body weight

- The batch 3: extract at the dose of 5000 mg / kg of body weight.

After administration of the substance orally to the different batches, the young female rats were observed individually during 14 days. These observations concerned the behavior and general condition of young rats.

The DL 50 is lower than the trial dose (2000 mg / kg or 5000 mg / kg), if at least three animals die.

The DL50 is superior to the trial dose (2000 mg / kg or 5000 mg / kg), if at least three animals survive [11].

Preparation of doses to be administered

The determination of the various weight concentrations to be administered was made according to the following formula:

Cp = (Pm x D) / V

Cp: weight concentration (g / ml)
Pm: middleweight of animals (g)
D: Trial doses (2000 mg / kg or 5000 mg / kg)
V: Volume of administration (2 ml)

The weight concentrations to be administrated are calculated as following (table 1)

Table-1: distribution of animals in batches and determination of doses to be administered

	Batch 1: distilled water 2 ml / 100g of body weight	Batch 2: extract at the dose of 2000 mg / kg of body weight	Batch 3: extract at the dose of 5000 mg / kg of body weight
Pm: middleweight of animals (g)	163	178	174
Dose to be administrated Cp (g/ml)	-	178	435

Anti-inflammatory activity

The protocol of the anti-inflammatory activity was made according to the method of the induction of the edema of the right posterior paw of the rat induced by the carrageenan [12]. Females rat were fasted for 10 to 12 hours before the test. The initial diameter (D0) of the right posterior paw of every rat was measured, one hour before the test with a caliper.

Rats were distributed in 4 groups of 3 rats according to the weight and the following doses was administrated:
- Batch 1 (negative control): rats received distilled water;
- Batch 2 (positive control): rats received orally dexamethasone in the dose of 10 mg / kg
- Batch 3: rats received orally an aqueous solution of the extract to the dose of 250 mg / kg
- Batch 4: rats received orally the extract in the dose of 500 mg / kg.

One hour after force-feeding, 0,10 ml of solution of carrageenan 1 % (prepared with water for injectable preparation) was injected into every rats under the plantar aponeurosis of the right posterior paw of the rat. The measure of the diameter of the paw was made every hour, till the fifth hour. For every female rat the swelling of the right posterior paw was measured with a caliper and the percentage of inhibition of the swelling of the paw having received the carrageenan was calculated compared with the diameter of the healthy right posterior paw according to the formula

$$\% \text{ Inhibition} = \frac{(D_t - D_0) \text{ control group} - (D_t - D_0) \text{ group treated}}{(D_t - D_0) \text{ control group}} \times 100$$

D_t = diameter of the right posterior paw at time t
D_0 = diameter of the right posterior paw at time 0.

Content in total phenols:

The content in total phenols was determined according to the method recommended by Abdelaziz Merouane and al. [13] which uses the reactive of Folin-Ciocalteu and the acid ascorbic as standard. A volume of 500 µl of reactive of Folin-Ciocalteu and 450 µl of distilled water were added to a tube containing 50 µl of extract with strong agitation. After 3 minutes, 400 µl of Na2CO3 (75 g/l) were added. The tube was incubated in 25 ° C and in the darkness during 40 minutes. The absorbance was read at 760 nm with a spectrophotometer Uviline 9100.

The content in phenolic compounds of the extract was determined from the curve of calibration of ascorbic acid and the results are expressed in mg ascorbic acid equivalent per gram of dry extract (mg EAA/g DE).

Antioxidizing activity

The anti-radical activity of extracts was determined by the method of reduction of the radical free of the DPPH (1,1, diphenyl-2-picrylhydrazyl). A solution of DPPH was prepared by solubilization of 2,4 mg of DPPH in 50 ml of ethanol. A volume of 50 µl solutions of extracts and the reference antioxidant, the ascorbic acid was added in 1950 ml of DPPH. The mixture was left with the darkness during 2 hours and the discoloration comparing with the control containing only the solution of DPPH, was measured in a spectrophotometer at 517 nm against ethanol as blank.

The control contains all the reagents excepted the sample to be tested, which has been replaced by an equal volume of ethanol [14,15]. The percentage of trapping of the radical DPPH was calculated by the following formula:

% trapping = [(Absorbance of control-Absorbance of extract)/Absorbance of control] × 100

The SC50 (scavenging concentration 50 %) allowing to calculate the concentration of necessary extract to trap 50 % of the radicals DPPH is graphically determined by linear regression. The effective concentration (CE50) is the necessary concentration to reduce the initial concentration of the DPPH with 50 % expressed in mg antioxidant/g DPPH (mg AO/g DPPH) or mg Antioxidant/mol DPPH (mg AO/mol DPPH) for the pure molecules [16].

$$CE_{50} = SC_{50}/C_{DPPH}$$

The anti-free radical power (PA) is inversely proportional to the effective concentration

$$CE_{50} : PA = 1/CE_{50}$$

Statistical analysis

The quantitative data were presented in the form of average ± standard deviation (SD) in graphs and tables. The orderly analysis of the variance (ANOVA) in one factor was used to compare the averages the groups. The post hoc test of Newman-Keuls was used to make the multiple comparisons. The threshold of meaning was fixed to P-value < 0,05.

RESULTS

Extraction

The yield (R) on aqueous extraction by maceration of the powder of *Arthrospira platensis* is 50,46 %. The aqueous crude extract was a powder of slightly black blue color (table 2).

Table-2: Yield on aqueous extraction of *Arthrospira platensis*

seaweeds	Weight of powder (g)	Weight of crude extract (g)	Yield on extraction (%)
Arthrospira platensis	200	100,93	50,46

Phytochemical study

The phytochemical characterization of the aqueous extract of *Arthrospira platensis* showed the presence of tannins, saponosides, terpenes, alkaloids and flavonoids (table 3).

Table-3: Results of the phytochemical screening of *Arthrospira platensis*

Secondary metabolites	Aqueous extract of *Arthrospira platensis*
Tannins	+
Anthraquinones	-
Anthocyanes	-
Coumarines	-
Flavonoids	+
Saponosides	+
Terpenes	+
Alkaloids	+
Sucres réducteurs	-

- : none; + : present

Microbiological quality control:

The results of the microbiological analysis of the spirulina show no presence of total coliforms (CT), of faecal coliforms (CF), yeasts and molds, and of salmonellas (table 4).

Table-4: Microbiological quality control of *Arthrospira platensis*

Sample	Types of microorganisms			
Arthrospira platensis	Total coliforms	Fecal coliforms	Yeasts and moulds	Salmonellas
	None	None	None	None

Acute toxicity

The observation after administration by oral route of a single dose of the aqueous extracts of *Arthrospira platensis* during 14 days of test showed the variations of the physiological parameters summarized in the table 5.

Anti-inflammatory activity:

The edema induced by the carrageenan was significantly reduced by the aqueous extract of *Arthrospira platensis* (table 6; figure 1)

Table-5: variation of some parameters during the evaluation of acute toxicity

Limit tests	Dose mg/kg	Mobility	Tremor	Reaction to noise	Aspect of stools	Coat	Death
Arthrospira platensis	2000	Yes	None	Yes	Normal	Normal	None
	5000	Yes	None	Yes	Normal	Normal	None

Table-6: Effect of the aqueous extract of *Arthrospira platensis* on the edema of the right posterior paw of the female rat induced by the carrageenan

Substances	Dose (mg/kg)	t_0	30 mn	1h	2h	3h	4h	5h
Distilled water	-	4,51 ± 0,28^{a}	6,95 ± 0,46^{b}	7,05 ± 0,15^{b}	7,28 ± 0,21^{b}	6,73 ± 0,20^{b}	6,68 ± 0,19^{b}	6,17 ± 0,37^{b}
Dexaméthasone	10	4,43 ± 0,15^{a}	5,80 ± 0,35*^{b}	5,65 ± 0,33*^{b}	5,12 ± 0,25*^{b}	4,70 ± 0,20*^{b}	4,70 ± 0,22*^{b}	4,65 ± 0,38*^{b}
		(ns)	(43,84)	(51,97)	(75,30)	(87,96)	(87,69)	(86,87)
Aqueous extract of *A. platensis*	250	4,26 ± 0,15^{a}	6,48 ± 0,29^{b}	6,45 ± 0,52^{b}	6,47 ± 0,31$^{\#b}$	6,18 ± 0,63$^{\#b}$	6,14 ± 0,46$^{\#b}$	5,08 ± 0,23$^{\#b}$
		(ns)	(8,90)	(13,81)	(20,45)	(13,53)	(13,38)	(10,10)
Aqueous extract of *A. platensis*	500	4,60 ± 0,20^{a}	6,63 ± 0,38^{a}	6,58 ± 0,34^{b}	6,48 ± 0,35$^{\#b}$	6,13 ± 0,31$^{\#b}$	6,10 ± 0,18$^{\#b}$	5,18 ± 0,26$^{\#b}$
		(ns)	(16,43)	(21,71)	(31,92)	(30,83)	(30,76)	(28,28)

The data are presented in the form of average ± standard deviation (SD). Every value represents an average of 3 animals (n = 3). The values in brackets represent the percentage of inhibition. The test of Newman-Keuls was used to make the comparisons with regard to the control. ns = not significant. Asterisks (*, ***) indicate a significant difference respectively at p < 0,05, p < 0,0001 comparing to distilled water. The sharps (#, ###) indicate a significant difference respectively at p < 0,05, p < 0,0001 comparing to the dexamethasone. For the same line, numbers (a, b) carrying the same letter are not significantly different (The threshold of meaning was fixed to P-value < 0,05).

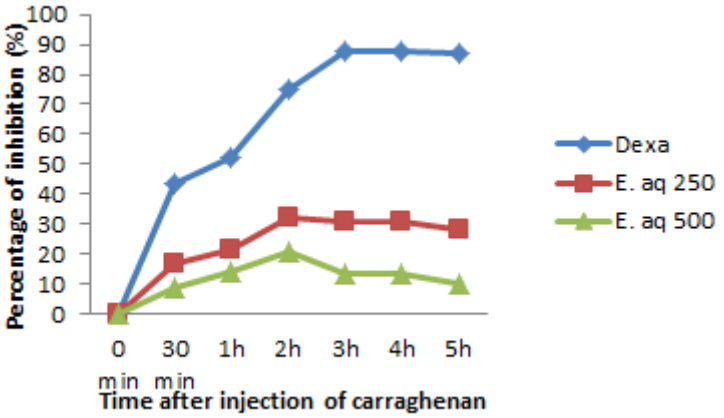

Fig-1: Curve of evolution of the oedema induced by the carrageenan.

The data are presented in the form of average ± standard deviation (SD). Every point represents an average of 3 animals (n = 3).

The oral administration of 10 mg/kg of dexamethasone one hour before the induction of the edema causes a significant decrease (p 0.05) of the edema of the leg compared with that of the rats of the

control group. The effect of inhibition of the dexamethasone was showed from the first hour which followed the injection of the carrageenan, the inhibition was maintained almost at the same level during 5 hours, with a percentage of inhibition about 80 %. The pretreatment of rats by the aqueous extract of Arthrospira platensis in the doses of 250 and 500 mg/kg leads to a significant reduction (p 0.05) of the inflammation comparing with the rats of the control group. The inhibitive effect of the aqueous extract in both doses is visible only from the second hour, with a percentage of maximal inhibition respectively of 20,45 % and 31,92 % with a significant difference (p 0.05) comparing with the rats of the reference group.

Content in total phenols: the results show that the average content in total phenols of the aqueous extract is 0,647 mg EAA / g DE (equivalent of antioxidant activity/g of dry extract) while that of ascorbic acid is 0,352 mg EAA / g DE. The curve of calibration of ascorbic acid and the content in total polyphenols of the aqueous extract of Arthrospira platensis and the ascorbic acid are represented in the Figures 2 and 3.

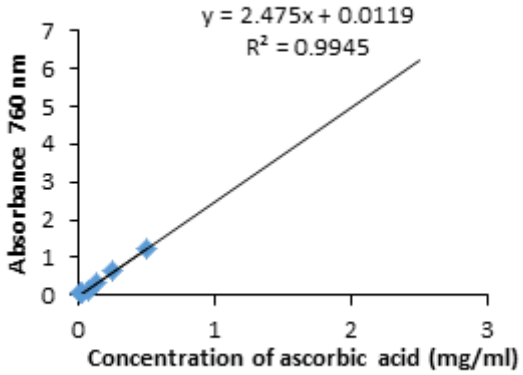

Fig-2: Curve of calibration of the ascorbic acid

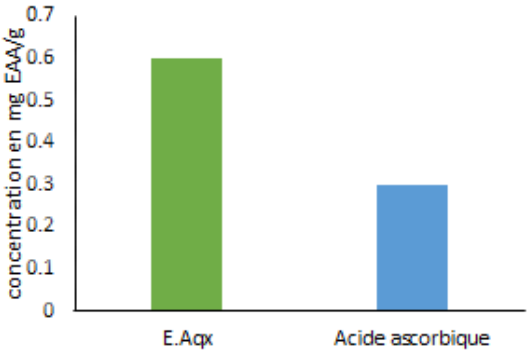

Fig-3: Content in total polyphenols of the aqueous extract of *Arthrospira platensis* and the ascorbic acid

Antioxidant activity: the antioxidant activity of the aqueous extract of Arthrospira platensis, shows an IC50 % (0,056 mg / ml), who is higher than that the one recorded for the ascorbic acid, 0,033 mg / ml (Figure 6). The ascorbic acid has an anti-radical power (1,496) upper to that of the aqueous extract (0,888) (table 7)

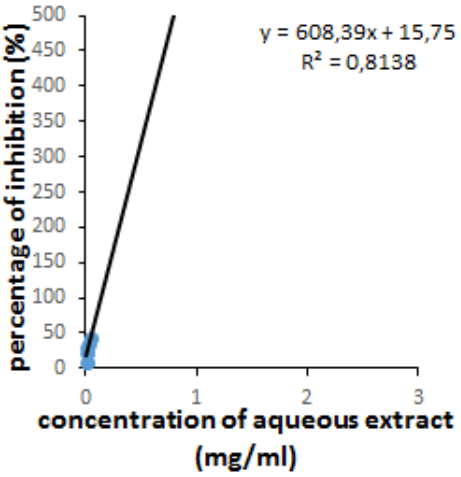

Fig-4: Percentage of inhibition of the aqueous extract of *Arthrospira platensis*

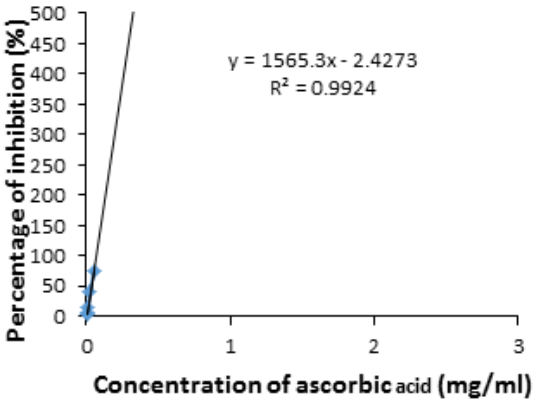

Fig-5: Percentage of inhibition of the DPPH according to the various concentrations of the ascorbic acid.

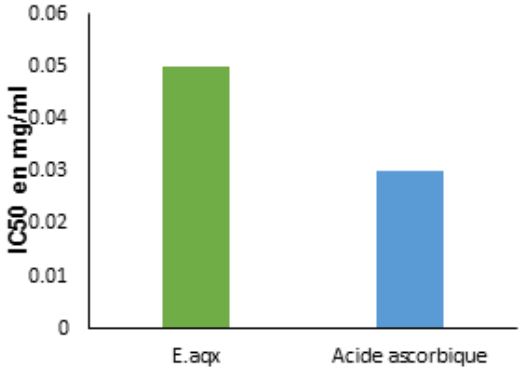

Fig-6: IC_{50} of ascorbic acid and the aqueous extract of *Arthrospira platensis*

Table-7: Anti-radical activity of the aqueous extract of *Arthrospira platensis*

	IC_{50} (mg/ml)	EC_{50} (mg/g DPPH)	Anti-radical activity
Ascorbic acid	0,033	0,668	1,497
Aqueous extract	0,056	1,125	0,888

DISCUSSION

The present study allowed to highlight the pharmacological properties of the aqueous extract of A.

Platensis and to characterize the chemical groups which contains the extract.

The anti-inflammatory activity is realized by the test of the carrageenan. This phlogogenic agent led on the leg of the rat an edema considered as a characteristic sign of the inflammation and a very important parameter in the evaluation of the anti-inflammatory activity of several compounds [17]. The injection of the carrageenan in the leg provokes a biphasic inflammatory answer of which the initial phase which lasts approximately 1h30 min after the injection of the carrageenan, shows itself by the liberation of the serotonine, the histamine, the bradykinine. The second phase which intervenes later is due to the biosynthesis of prostaglandins [18, 19]. Indeed the injection of the carrageenan leads an increase of synthesis of the ARNm of the cyclooxygenase 2 (COX-2) which causes a rise of the concentration of this enzyme with a peak after 1h. This rise is accompanied by an increase of the synthesis of prostaglandins (PGs), essentially the prostaglandin E2 (PGE2) (maximum after 2 h) involved in the processes of the pain and the inflammation [20, 21]. Steroidal anti-inflammatory drugs (AIS) as the dexamethasone act from the first hour, because it interacts directly with the DNA. Its effect is in connection with several molecular ways (pro-inflammatory cytokines, phospholipase, COX) generally through the nuclear factor of transcription (NF-KB) [22, 23]. The results show that the anti-inflammatory activity is significantly inhibited ($p<0,05$), for doses of spirulina between 250 and 500 mg / kg giving evidence that the spirulina has an inhibitive effect on the inflammation.

The works of Duerring and et al. [24] specify besides that the monomers of phycocyanine have α and ß would be responsible for the anti-inflammatory effect of the spirulina. These authors show in their works that the isolated and cleansed phycocyanine has it anti-inflammatory role. Romay and al. [25] in their works on rats show it very well by obtaining significant inhibitions between 26,5 % and 43,7 % for doses of extracts of phycocyanine respectively for 100 and 200 mg/kg. On the other hand the obtained respective inhibitions are clearly higher than those of the present study (20,45 % and 31,92 %).

Total phenols play an important role in the protection against some diseases, because of their possible interactions with numerous enzymes and also towards their antioxidizing properties. These compounds also modulate the activity of several enzymes or the cellular receivers [26]. In the aqueous extract of Arthrospira platensis the content in total phenols (0,64 mg EAA / g DE) is lower than the value (0,79 mg EAG / g DE) found by Boutalbi [27]. The polyphenolic contents vary qualitatively and quantitatively from a specie to the other one; it can be attributed to several factors:

- Climatic and environmental factors: the geographical zone, the drought, the ground, the attacks and the diseases, etc.;

- The genetic heritage, the period of the harvest and the stage of development of the plant [28];

- The method of extraction and the method of quantification can also influence the estimation of the content of total phenols [29].

The reactive oxygenated species (ROS) intervenes in the physiopathology of the diseases with inflammatory component (cancer, diabetes, atherosclerosis, arthritis, infectious diseases). The ROS leads to the liberation of cytokines (TNF α, IL 1β, IL 6) and the activation of the proinflammatory enzymes (cyclo-oxygenases, lipoxygenase, inductible nitrogen mono oxide synthase) occurring in the inflammatory process [28]. The results of the present study show that the aqueous extract of Arthrospira platensis possesses an anti-radical activity (0,888), but not as much as the ascorbic acid which possesses an anti-radical power higher (1,497). Similar results of the anti-radical power of the aqueous extract of Arthrospira platensis by the method of trapping of the free radical DPPH were obtained by Boutalbi [27] who had a low value of IC50 (0,042 mg / ml), inversely proportional to the anti-radical power which is thus brought up. In the phytochemical analysis, the presence of the phenolic compounds, especially tannins and flavonoids could be one of the reasons of her antioxidizing activity.

The microbiological analysis of the spirulina shows no presence of total coliforms, of fecal coliforms, yeasts and molds, and of salmonellas. The absence of microorganisms is the result of good conditions of harvest, drying and preservation, and absence of contamination by feces. The same observations were brought back by the ITRAD (Chadian Institute of Agronomic Research for the Development) on the improved spirulina “Dihé" in 2006 and 2008 [30].

The values of DL50 were determined by the oral administration of the aqueous extract of Arthrospira platensis to rats. The values of DL50 were considered superior to 5000 mg / kg. Animals were observed during 14 days and no behavior disorder, nor deaths in the dose 2000 and 5000 mg / kg were observed between handled animals and untreated. The same observations were made by Krishnakumari and al. [31] in the dose 800 and 2000 mg / kg with the albino rats. Romay and et al. find the same observations in the dose 3000 mg / kg to mice and rats [25], as well as

Hutadilok-Towatana and et al. [32] in the dose 10 and 30 g / kg with mice.

The phytochemical characterization of Arthrospira platensis showed the presence of tannins, saponosides, terpenes, alkaloids and flavonoids. These results are similar to those of Boutalbi [27] who reported the presence of the same classes of chemical families found in Arthrospira platensis. The presence of its secondary metabolites could be responsible for the anti-inflammatory and antioxidizing activity.

CONCLUSION

The present study showed that the aqueous extract of A. platensis possesses anti-inflammatory and antioxidizing properties. The presence of the main chemical groups such as tannins, saponosides, terpenes, alkaloids and flavonoids would be responsible for noticed pharmacological activities. The DL 50 superior 5000 mg / kg of the extract shows its possible therapeutic use up to this dose, as well as the microbiological quality. These pharmacological properties explain the efficiency of the products of A. platensis used for the treatment of the inflammatory pathologies. The results of this study are a perspective of an application in the pharmaceutical industry in the production of anti-inflammatory and antioxidant medicine, as well as a potential utility in the food industry as food complement.

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