Chemical Composition and Potential Application of *Spirulina platensis* Biomass

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Abstract: Submerged batch cultures, Semi -pilot scale cultivations and Outdoor biomass production were performed to increase *Spirulina platensis* biomass which is naturally grown in El Khadra lake water body. Comparing *Chlorella vulgaris* and *Spirulina platensis* showed higher protein contents of *Spirulina* as it reached 64 % (w/w) so, it may be used in agriculture as a nitrogen biofertilizer and as an animal and fish growth promoter. Bio-chemical analysis of *Spirulina* biomass showed presence of 17 amino acids, 10% (w/w) carbohydrates, 8 %(w/w) fibers and 8 %(w/w) lipids. *The biomass of Spirulina* contained 0.04 ppm Mg, 0.3 ppm Ca, 0.16 ppm Mn, .0.8 ppm Fe, 0.16 ppm Zn, 11.3 ppm Na, 0.003 ppm Se and 5.6 ppm K. It also contained 1 ppm Cu, 0.04 ppm Hg, 0.03 ppm Ni, 0.9 ppm Cr, 0.1 ppm Cd, and 0.6 ppm Co.
[Aly, M. S and Amber. S., Gad. **Chemical Composition and Potential Application of *Spirulina platensis* Biomass**. Journal of American Science 2010;6(12):480-488]. (ISSN: 1545-1003).

Key words: *Spirulina platensi,,* El Khadra lake, biofertilizer..

1. Introduction:

The mixotrophic culture might be used as an alternative to conventional photoautotrophic mass culture systems for production of high value pharmaceuticals by *Spirulina platensis*. Chen (1996) satisfactorily cultured *S. platensis* in a mixotrophic mode by using a mix of different sources of energy and carbon ; glucose at high cell biomass densities 10.24 (g/ L) for the production of phycocyanin. Under mixotrophic conditions, some microalgae are known to grow rapidly and to have a higher growth rate than under photoautotrophic conditions, Samejima and Myers (1958). The algae usually not only contain nearly every required vitamin and mineral, but also have the effect of increasing oxygen while reducing nitrogen and carbon. Lee *et al.* (1996) cultured *Chlorella sorokiniana* mixotrophically in an outdoor enclosed tubular photobioreactor reaching an optimum biomass productivity of 10.2 (g /L/ d) during the day and 5.9(g /L/ d)during the night using an initial glucose concentration of 0.1 M. The daily volumetric productivity of photosynthetic *Chlorella* cultures in a similar tubular photobioreactor was about 3 times lower, Lee and Low (1992). These values are the highest reported for outdoor conditions, Ogbonna *et al*. (1997). Chen and Zhang (1997) stated that growing *S. platensi* under mixotrophic conditions reported a productivity 2.4-fold higher than of photoautotrophic cultures. Mixotrophic cultivation is an alternative to photoautotrophic production of biomass. The growth rates and biomass concentrations increase, apparently due to a synergistic effect of light and the organic substrate, Cid *et al.* (1992). Mixotrophic cultivation is a dual limiting process in which low light intensities or low organic carbon substrate concentrations may limit cell growth; while high light intensities or high carbon substrate concentrations may inhibit cell growth, Chen (1996). The cellular concentrations of the photosynthetic products depend on the relative heterotrophic and photoautotrophic growth rates. At high cell concentrations, light becomes limiting and the contribution of the photoautotrophic growth to the overall growth rate is very low. Under this condition, both the protein and the chlorophyll contents of the cells are much lower than of those of the autotrophic cultures, Ogbonna *et al.* (1997).Heterotrophic growth of microalgae could eliminate the requirements for light, and thus may offer the possibility of greatly increasing the algal cell concentration and productivity on a large-scale; however, only a few industrial heterotrophic processes have been attempted to date**,** Chen and Johns (1995). This is probably because limited number of available heterotrophic algal species; increased potential of contamination by bacteria; inhibition of growth by organic substrates at low concentrations; and the inability to produce some light-induced products, such as pigments ,Chen (1996). Photosynthetic microorganisms are able to synthesis organic C, N, and P which allow treating wastewater and simultaneously producing useful biomass when intensive algal culture is employed in outdoor ponds as tertiary for the removal of waste residual compounds, Kim**,** *et.al* (2000). *S.platensis* cultivation is usually preformed in open ponds so that the solar energy is used to fix inorganic carbon. Since heterotrophic metabolism is faster than autotrophic, simple carbon source is used to sustain growth. However, heterotrophic metabolism tends to suppress

the photosynthetic activity, therefore a mixed heterotrophic and autotrophic (mixotrophic) culture could be preferable, Lee *et al*. (1992 and 1996).

The high content protein indicates relatively good amino acid profile and low metal content enabled the use of algal biomass as feed supplement. The use of micro- algae in industry encourage the development of better cultivation system in order to optimize the production of algae rich in active substances such as vitamins protein, amino acids,fatty acids and trace elements . This study aimed to evaluate the amino acid composition of corkscrew-shaped filament *S. platensis* microalgae grown in El Khafra lake water. This seaweed is also characterized by fast growth, (dividing three times a day) , Furthermore, the *Spirulina* has advantages over other seaweeds by having the pleasant taste and thus it is used in the preparations of *Spirulina* capsules or in foods such as beverages and pastes. *Spirulina* causes no problems for digestion and no toxicity to humans, in contrary to occur to other seaweeds such as *Chlorella* and *Scenedesmus*. *Spirulina* is also known for its antioxidant and hypocholesterolemic actions**;**Parikh *et al*. (2001); Mao *et al*.(2005) **;** Colla,. *et al. (* 2008)and Muthuraman *et al.* (2009*).*

2. Materials and Methods:

Micro-organisms:

Spirulina platensis and *Chlorella vulgaris* isolates used in this study were obtained from El-Khadra lake at Wadi El Natroun, Egypt, characterized by extreme conditions of pH 10.5 and salt concentration of 0.55 M,,(Aly ,2000).

Maintenance stock media:

Zarrouk's synthetic solid medium (Zarrouk, 1966), amended with 2% (w/v) agar was used for maintaining *S.platensis* N-8 medium; Vonshak(1986)used to maintain the unicellular green algae *Chlorella vulgaris.*

Inoculums preparation:

Spirulina stock cultures were propagated in 50 ml Zarrouk's synthetic broth medium at 30±2 °C under continuous illumination with fluorescent lights for seven days in 500 mL Erlenmeyer flasks; Colla *et al.* (2004). The inocula concentrations were determined by spectrophotometer using a standard curve. a calibrated at 560 nm ;Volkman(2008). Inoculation conditions of *C.vulgaris* were performed according to Mandalam and Polsson and (1998).

Batch culture condition.

The *S. platensis* batch culture was incubated_at agitation of 160 rpm, temperature, 30ºC and luminosity 5000 lux in 250 mL Erlenmeyer flasks containing 50 ml of both basal Zarrouk medium, Zarrouk, (1966), and El Khadra lake water. Fermentations media were sterilized at 120°C for 20 min. The stock cultures were transferred to outdoors 130-L photobioreactor earlier 24 hours after inoculation. Cultivation conditions of *C. vulgaris* were performed according to Mandalam and Polsson and (1998).

Semi -pilot scale condition:

The bioreactor was inoculated by the previously prepared locally isolated *S.platensis* and *C. vulgaris* with initial biomass concentration of 0.16 g/l, These cyanophytes were used to inoculate Zarrouk's synthetic media ,Zarrouk, (1966) ; N-8 medium ,Vonshak (1986) and El Khadra water respectively. Cultures were incubated at 30°C with 5000 lux/cm^2 and pH 10.5 for 7 days . Zarrouk's medium contained (g/L):$NaHCO_3$, 16.8; K_2HPO_4, 0.5; $NaNO_3$, 2.5; K_2SO_4, 1.0; NaCl, 1.0; $MgSO_4 \cdot 7H2O$, 0.2; $CaCl_2$, 0.04; $FeSO4 \cdot 7H_2O$, 0.01; EDTA, 0.08; H_3BO_3, 2.86 mg/L; $MnCl_2 \cdot 4H_2O$, 1.81 mg/L; $ZnSO_4 \cdot 7H_2O$, 220 µg/L; $CuSO_4 \cdot 5H_2O$, 79 µg/L; MoO_3 , 15 µg/L; and Na_2MoO_4, 21 µg/L; Cases *et al*. (2001)). All the reagents used were of analytical grade, obtained from Merck Chemical Co. (Darmstadt, Germany). Aeration was conducted using air pump according to Costa *et al.*, (2002), and was incubated for 7 days at 30°C under illumination with fluorescent lamps (5000 lux) with 12h light /dark photoperiod according to Vonshak *et al.,* (1982).

It was supplied with flow rate of 0.03% CO_2. (100ml /min) in a 3L batch fermentor of 2.5L working volume with air. Cultivation conditions of *C.vulgaris* were performed according to Mandalam and Polsson and (1998) .After seven days , the biomass was filtered through a 20-µm membrane, thoroughly washed with distilled water, and stored after freeze drying.

Outdoor *Spirulina* biomass production*:*

2 L Erlenmeyer flasks, containing 1.8 L of Zarrouk's nutritive medium (Zarrouk, 1966) were used as starter inocula. *S. platensis* were grown in water outdoor mass culture 130 L photobioreactor, Colla *et al*. (2004).

Harvesting the biomass

At the end of seven days, the algal biomass was aseptically filtered and washed with distilled water to remove the salts from the algal surface then, lyophilized and stored at 4-5◦C °until need according to ,Colla *et al*.(2004) or dried at 50° C. The dried *S.*

platensis was powdered and stored in plastic container till used.

Preparation of *Spirulina* aquous extract (SAE):

The air dried *S.platensis* cells (5 g) was mixed under cooling conditions with sterile dist. water. The slurry was filtered through 50 mesh polyester rope and centrifuged at 5000 rpm for 10min. The clear supernatant was taken and made up to 100 ml then, sterilized through Millipore membrane 0.25µ.This sterilized *Spirulina* aqueous extract (SAE) represents the stock sources used for chemical analysis.

Analytical procedures.

pH of El Khadra lake was determined directly in the field with scan 2 pH meter .

Determination of the protein content of the cultivated material according to Lowry *et al.* (1951).

Determination of amino acids The amino acid profile was analyzed in algal protein precipitate after HCl hydrolysis using LC3000 amino acid analyzer with a flow rate , 0.2 ml/min , pressure for buffer, 0-50 bar, pressure for reagent ninhydrin 0-150 bar, column temp. 50°C.

Total carbohydrates were determined as glucose according to Dubois *et al*. (1956).

Fibers contents of *S. platensis* were determined according to the standard method of A.O.A.C. (1980).

Lipids were determined according to Folsh *et al.* (1975) method using extraction mixture of methanol – chloroform mixture (1:1,v/v) at 28ºC for 24 hrs in darkness followed by filtration. The extract was mixed thoroughly with half volume of 0.9% NaCl solution and the organic phase containing fatty acids was separated. The solvent was evaporated under nitrogen and the lipid content was gravimetrically estimated.

Heavy metals and minerals were analyzed by atomic absorption spectrophotometer varian A220, NRC, Cairo.

Spirulina semi-pilot scale production studies:

100 mg axenic culture prepared from powder of the local *S.platensis*; 15 mg PVP (polyvinylpyrrolidone) ; 10mg magnesium stearate and 300 mg avicel .For all formulations with (5-20,w/w) PVP the mixture was compressed using single punch tablet machine , hardness tester, disintegration apparatus and Roche Frabilator Erweka (GmbH. Frankfurt) in the machine setting were adjusted to produce *S.platensis* pellets having approximately the same hardness and weight .

3. Results and Discussion:

Protein content of *S. platensis* and *C.vulgaris* grown in El Khadra Lake and on basal synthetic media.

Fig(1) shows that the bluish-green seaweed *Spirulina* is characterized by its high protein content (64.0,and 58,0)% on dry weight basis when grown in Zarrouks, and El Khadra lake water ,respectively compared to *C. vulgaris* grown in N-8 medium Vonshak (1986)and El Khadra lake water .So, further studies will be focused on *Spirulina* .As a cyanobacterium, *S. platensis* does not contain a heterocyst necessary for nitrogen fixation. Thus, it must absorb nitrate from the media, and cultivation requires substantial inputs of soluble nitrogen. Exclusive use of sodium nitrate is essential for biomass production. *S. platensis* grown on basal medium because of its high solubile nitrate.

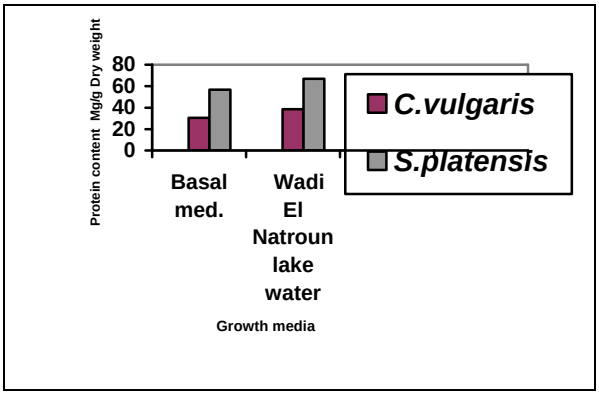


Fig.(1)Protein content of *Chlorella* and *Spirulina* grown in basal media and Waadi El Natroun Khadra lake body water.

The Biochemical analysis of *S.platensis*

Nitrate assimilation involves its uptake and sequential reduction by nitrate reductase (NR) and nitrite reductase (NiR) into ammonium ions, which are then incorporated into amino acids mainly by the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway. In higher plants, these enzymes are induced by nitrate and regulated by light, hormones, sugars and carbon and nitrogen metabolites, Raghuram *et al,* (2006). The genes involved in these reactions have been cloned from many plants and mutants and transgenic lines are also available,Lochab *et al.* (2007). *Spirulina* contains at least 10-fold higher protein than rice, indicating a correspondingly high capacity for nitrate utilization,(Goto *et al.* 1998,;Kronzucker *et al.* 2000;Ali *et al.*(2007). However, its biochemical basis is not known ,Vonshak (1997), except nitrate induction by NR and its inhibition by nitrite and

ammonium ions ,Jha *et al*., (2007), Characterization of NiR (Yabuki *et al*., 1985; Ali *et al.* 2008), and regulation of nitrate assimilation by calcium and phosphate were reported ,Singh and Singh (2000). Wong and Chan (1980) found that the protein content of sewage grown *Spirulina* was 45.6 %and fat was 14.7% of the total dry weight

Results obtained in table (1) revealed the *Spirulina* high crude protein content which amounted 64.00(% w/w) on Zarrouk medium. Crude fat was 8% (w/w); 10%(w/w) total carbohydrate content;8%(w/w) fiber .Wong and Chan(1990)revealed that *C.vulgaris* and *S.bijuga* have a fiber content of 5-8%(w/w)and 10%(w/w) minerals. The protein malnutrition is a public health problem that has affected a large proportion of the world population for many years, especially in the developing countries indicates tremendous importance of this alga in nutritional, industrial and environmental biotechnology, Vonshak (1997). However, it is not clear how the organism steers its nitrogen metabolism to produce so much protein. This alga is a nitrate-utilizing, non-nitrogen fixing and photosynthetic organism. The nitrate-utilizing ability of *Spirulina* has been exploited in the decontamination of nitrate-polluted waters and effluents (Kim *et a*l., 2000; Lodi *et al*., 2003).

Amino acid profile of *S. platensis*

Seventeen amino acids were detected in *S. platensis* grown on Zarrouks growth media as table (2) shows that *S. platensis* contains wide spectrum of amino acids . Glutamic acid was the most common amino acid of the dry matter of *S. platensis* followed by aspartic acid. Isoleucine was the most abundant essential amino acid as indicated in table (2).The phenyl aalanine is also present in comparatively high doses, therefore, people with phenylketonuria should avoid *Spirulina*.

The high protein and amino acid content of the algae grown on agriculture drainage water could be attributed to the availability of essential elements in quite high amounts as well as the tendency of algae for bioaccumulation and incorporation of these element into their macromolecules .El Adel *et al*. (2003) reported that salinity ,inorganic N and P of agriculture drainage water resulted in higher protein content in *Spirulina* especially due to the lack of organelles and intracellular transport constraints. The contents of proline and sulfur containing amino acid :methionine and cystine of the algae is low remarkably. The results reported that amino acids aspartic, serine, alanine, leucine, and glycine collectively amounted 50% of the detected amino acid content while methionine, cystine, tyrosine, and histidine collectively amounted less than 20 %.

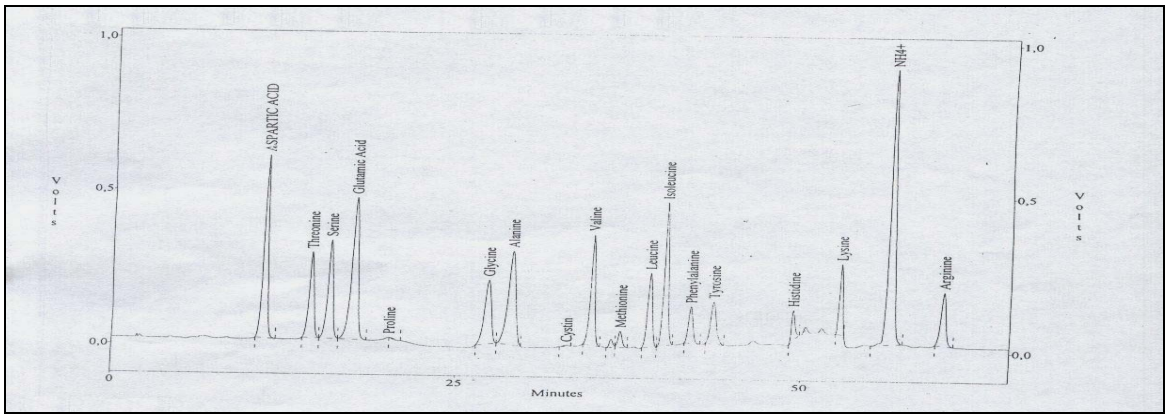
The amino acid profile of *S. platensis* biomass is a presented in Fig (2).

Table(1). The Biochemical analysis El Khada lake of *S. platensis* biomass grown in Zarrouks basal medium.

Component	Concentration (%w/w)
Crude protein	64.00
Fat (lipids)	8.00
Total carbohydrate	10.00
Fiber	8.00
Minerals	10.00

Table (2).Combined amino acids in the biomass of *S .platensis* grown on Zarrouk's basal medium .

Eessential Amino acid	(µg/mg) DW.	Non essential Amino acid	(ug/mg)DW.
TTheorinnine	16.068	Alanine	29.564
PPhenylalanine	11.537	Serine	16.109
A Arginine	23.359	Tyrosine	16.765
Histidine	7.147	Proline	8.767
Luecine	19.485	Glycine	
Isoluecine	37.325	Glutamic acid	52.159
Lysine	13.460	Aspartic acid	38.186
Valine	22.394	Cystine	1.1
Methionine	4.840		



Fig(2)-HPLC- chart amino acid profile of combined amino acids of. *S.platensis.*
(Amino acids in order from left to right)Aspartic-Theorinnine- Serine- Glutamic acid- Proline- Glycine- Alanine- Cystine Valine – Methionine-Luecine- Isoluecine- Phenylalanine- Tyrosine-Histidine- Lysine.

Minerals in *S. platensis*

Table (3) shows that, *Spirulina* might concentrate ions found in its environment. The natural El khadra lake waters *Spirulina* are so saturated with minerals originating from ancient soils and mountains while *Spirulina* favors to live in this aquatic environment no other plants can live there. Because *Spirulina* prefer to thrives in such alkaline waters, it incorporates and synthesizes many minerals and derivative compounds into their cells. The absorbed minerals are transformed into natural organic forms by *Spirulina.* Minerals become chelated with amino acids and are become more easily assimilated by the body .The biomass of *S. platensis* contained 0.04 ppm Mg, 0.3ppm Ca, 0.16 ppm Mn,0.8 ppm Fe,0.16 ppm Zn,11.3 ppm Na,0.003pppse,and 5.6pppK. Potassium is a crucial mineral that regulates body electrolyte balance, Beck (2000). Its deficiency can cause heart arrest, hypertension, adrenal exhaustion and muscular collapse. Calcium is especially important to bone and dental health and also in neural transmissions to the muscles; Aloia, *et al.(*1996). Zinc is the pivot point of over thirty vital enzymatic reactions, with profound effects on mental health, skin tone, prostate function and healing capacity**, Gonzalez**(2009). Magnesium deficiency can lead to spasmodic muscle disorders, including cardiac irregularities. This element helps in assimilation of C, B vitamins and proteins; Sawka and Montain(2000). Manganese at concentration of 0.16 (ppm) activates enzyme systems, along with zinc. It promotes the activity of neurotransmitter acetylcholine, and helps stabilize blood sugar ,Takeda(2003). Selenium was originally believed to be a toxic heavy metal, but now known to be necessary for health. It retards aging, harmful oxidation and free radical formation, reduces and improves cardiac efficiency. Selenium is an essential trace mineral that functions as an antioxidant and promotes a healthy immune system; Cases *et al.* (2001), and Tsavachidou *et al*. (2009). Iron is required in remarkably small amounts .It promotes formation of hemoglobin, the oxygen-carrying blood pigment found in healthy red blood cells. Iron deficiency is most common among women in their reproductive years, Steinberg (2001).

Table (3). The minerals content of *S. Platensis* .

Mineral	Conc. (ppm)	Mineral	Conc. (ppm)
Mg^{+2}	0.04	Zn^{+2}	0.16
Ca^{+2}	9.3	Na^{+}	11.3
Mn^{+2}	0.16	Se^{+4}	0.003
Fe^{+2}	0.8	K^{+}	5.9

Heavy metals *in S. platensis:*

The UN-FAO recognizes *Spirulina* as a potential weapon against malnutrition for the third world and has sponsored safety studies since the early 1980. Table (4) shows the heavy metals content of *Spirulina*. Phytoremediation potential of *S. platensis:* resemble *Chlorella* which known to bind to the heavy metals .Biosorption and toxicity probably most concern is *Spirulina's* ability to absorb and concentrate heavy metals such as lead and mercury if they are present in its environment The biomass of *S. platensis* contained 1.00 ppm Cu, 0.04 ppm Hg, 0.03 ppm Ni,0.90 ppm Cr,0.10 ppm Cd,and 0.6 ppm Co.. However, *Spirulina*-associated hepatotoxicity and reactions from heavy metal contamination are possible. *Spirulina* is considered nontoxic to humans at usual levels of consumption .This justify the use of alga biomass in nutrition if necessary; however, information is limited as insufficient clinical data to guide dosing of *Spirulina* for therapeutic effect.

Table (4).The Heavy metal content of *S. Platensis*

Heavy metal	Conc. (ppm)	Heavy metal	Conc. (ppm)	Heavy meta	Conc (ppm)
Cu^{+2}	1.00	Hg^{+2}	0.04	Ni^{+3}	0.03
Cr^{+2}	0.901	Cd^{+2}	0.10	Co^{+3}	0.60

Therapeutic *Spirulina* pellets:

Because of the high cost of extraction of polyunsaturated fatty acids and other constituents from *Spirulina* ,it seems that the best way is to use *Spirulina* by direct consumption as a nutritional supplement,Kapoor and Mehta (1993), *Spirulina* can be used either as a food supplement or taken in capsule form.Capsules appeare to be the preferred form at present, Colla *et al*. (2004).

Table (5) shows that 5 % (w/w) poly vinyl pyrrylidone (PVP) is the most suitable for capsules formation. *Spirulina* is sold as a feed additive for aquaculture and as a dietary supplement It has a long history of use as food and it is the nature's richest and most complete source of organic nutrition. The concentrated nutritional profile of *Spirulina* occurs naturally, so it is ideal for those preferring a whole food supplement to artificial nutrient sources. *Spirulina* has a unique blend of nutrients that no single source can provide. It has been labeled as a powerful food, rich in proteins, carbohydrates, polyunsaturated fatty acids, sterols and some more vital elements like calcium, iron, zinc, magnesium, manganese and selenium. It is a natural source of vitamin B12, vitamin E, ascorbic acid, tocopherols and whole spectrum of natural mixed carotene and xanthophylls phyto-pigments (Chamorro *et al*., 1996; Pinero Estrada *et al* .2001; Chamorro *et al.,* 2002, Islk *et al.,* 2006, and Al Attat 2010).*Spirulina* is also used as a feed ingredient for pigmentation of ornamental fish, especially gold fish and fancy red carp. *The spirulina* is reported as a potent anti-cancer (Ismail *et al.,* 2009), hypocholesterolemic and hypolipidemic (Colla *et al.,* 2008), antidiabetic (Muthuraman *et al.*, 2009) as well as for health improvement (Annapurna *et al.,* 1991; Cingi *et al.* 2008, and Al Attar, 2010).

Despite considerable progress in medical therapy, there is no satisfactory drug to treat kidney stones. So, capsules could be used for antilithiatic activity of *Spirulina* . In addition, the incidence of kidney stones has been increased in most societies in the last five decades, especially in association with economic development. In spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of nephrolithiasis. Recently, there is increasing evidence that many healthy natural food and medicinal herbal and supplements have the potential to become valuable complementary therapy in the treatment of various renal disorders and in the protection against iatrogenic nephrotoxicity. The administration of *Spirulina* solution to rats with ethylene glycol induced nephrolithiasis reduced and prevented the growth of kidney stones, renal and hepatic impairment can be concluded that the food supplementation with *Spirulina* has a beneficial effect on nephrolithiasis induced by ethylene glycol, Al-Attar(2010). The *Spirulina* components which are responsible for these therapeutic properties are thought to be compounds with antioxidant abilities such as polyunsaturated fatty acids, phycocyanin and phenolics ,Colla *et al*. (2004),and Islk *et al.(*2006).

Table (5).Physical characteristics of *S. platensis* pellets formulation.

PVP conc.(%)	Weight mg(±CV)	Thickness(±SD)	Friability(%)	Hardness(kg)	Disintegration time(min)
5	300±1.70	0.321±0.80	1.05	6.43	0.22
10	338±1.90	0.351±0.80	0.99	5.92	1.73
15	278±0.50	0.333±1.21	0.06	5.77	2.76
20	302±1.75	0.322±1.53	0.05	6.99	6.28

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10/26/2010