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PHARMACOLOGICAL AND BIOLOGICAL PROPRIETES OF A MIXTURE OF DATE POWDERS (MECH-DEGLA AND SPIRULINA

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Abstract

Several research tasks show the importance of use of thedryMech-Degladatesvariety in the preparation of various foodstuffs. On the other hand, the Spirulina, qualified as a protein concentrate source whose phycocyanin (the Spirulina blue green pigments) has been known according to its several pharmacological properties. The objective of this study isto develop a new functional foodstuff containing Mech-Dglapowders date and Spirulina.The obtained mixed product has been analyzed and biochemical evaluated and the results are :1) as regards date: it is rich especially in sugars (TSS=60 ± 0,4% n=3) with low proteins content (2,1 ± 0,4%; n=3) compared to the Spirulinawhich is rich in proteins (61,745 ± 0,0353; n=3), bioactives substances asphycocyanin (1,7 ± 0,0015 g/100g ms; n=3); total chlorophyll (1,0463 ± 0,0946 %; n=3)and ashes (9,41 ± 0,03%; n=3); 2). The two powders are revealed, under the experimental conditions, have good microbiological qualities. Moreover, the three extracts of xanthophylls, ß carotène and phycocyanin of the Spirulina are characterized by a:1)bactericidal action against S. aureus and P. aeruginosashowing intermediate zone of inhibition (14, 10,66 mm); 2) fungicidal action versusA.nigerwith inhibition zone of 10mm. It is noticed that, a solution of 2mg/ml of each extract is largely enough to inhibit the tested microorganisms.The biological activity of the extracts of the Spirulina with respect to the pathogenic agents is related to the composition of the essential fatty acids (C14: O (46,02%), C18: 2 (13,11) C18: 3 (9,53%), C18:2 (13,11 %) and with others volatile compounds such as polyphenols (5,1325%EAG). Another parameter considered to be useful is the time of disintegration of the immersed tablets in distilled water which is similar to that found in other research relating to some pharmaceutical tablets. In the same way, low escape velocity of the phycocyanin (16% in 45 mn) which is a significant factor relatively to the therapeutic properties since low dose of this active ingredient would be more effective like an anti inflammatory substance.

Keywords*: spirulina, pigments, phycocyanin, dates, pharmacological properties*

1. Introduction

Algeria is a date producer country (*Phoenix dactylifera L)*with an annual production of more than 500 000 t. The most significant part of this quantity constitutes common dates. Those are dry varieties with commercial low value. And, the production of common dates in Algeria reached 244577 t (SAA, 2011). Generally, the date fruit constitutes a principal component of the food in several countries (Reynes and *al.,* 1994; Al-Shahib and Marshal, 2003). The common dates such as *Mech-Degla* havean undeniable economic importance (Brac of Perriere, 1988). These dates, are known according to their dry consistency and constitute a true concentrate of essential sugars and nutriments like fibers, the B group vitamins, iron and potassium elements.They contain also beta carotene and amino acids. However, the surplus of the production of many known varieties poses a marketing problem for the farmers. They are generally destinated for cattle feeds which is likely to weaken the phoenicicole system (Acourene and Tama, 1997). In parallel, Spirulina is regarded as a nonconventional food resource being able to contain up to 70 % of proteins; it is rich in rock salt, trace elements and many vitamins (B1, B2, B12, E...) (Salland *al.* 1999). Spirulina which is a blue-green microalga was proposed in the human consumption (FAO) by several scientists and nutritionists because of its culture facility, its high productivity and its low production cost.

The objective of this study aims a development of a new functional foodstuff containing dates powders and Spirulina. The date powder is known by its wealth of sugars and its low content of proteins.On the other hand, the qualified Spirulina as being richest foodin the world, is considered as a true protein concentrate. But it is observed that, its strong savour alas is accepted sometimes with difficulty by certain consumers. In addition to chlorophyll, the Spirulina contains the phycocyanin which is a known pigment with interest antioxidantactivity (Chen and Wong, 2008). Indeed, various studies showed the role of the Spirulinain the prevention of many pathologies like cancer, cardiovascular diseases and ageing premature (Reddy and *al.,*2000; Girardin-Andréani, 2005). Therefore, there are several applications of the Spirulinafor the human consumption as instantaneous noodles for children (Xu, 1993); drinks (Zengand Liang, 1995) and Japanese compressed form (Yamaguchi, 1997).

The aptitude of the *Mech-Dgla*dates to undergo a complementary drying (thermal or with microwave) for obtaining powders then food tablets was already underlined (Amellal and Benamara, 2008; Benamara and *al*.2009). On another plan, it was revealed the suitability of date syrup like binder and aromatizing in the process of obtaining pharmaceutical tablets (Alanzi, 2010). Then, the comprehension of the behavior of the powder is imperative to lead a final product withbest physical quality (Chen and Li, 2009). Accordingly, we try to formulate food tablets containingpowders from *Mech-Degla*dates and Spirulinafrom Burkina-Faso origin. The physicochemical and microbiological characterizations of these powders were carried out and alsoantibacterial activity of their various freeze-dried extracts with respect to the pathogenic bacteria was studied.

2. Materials and Methods

2.1. Material plant

2.1.1. Dates

The dry *Mech-Degla*variety of common dates is used in the present research. This variety harvested from Biskra and bought in the local market of BoumerdèsAlgerian city during the period being spread out of the 21-03 to 25-04-2010.

2.1.2. Spirulina

Spirulina (*Spirulinaplatensis)*used in this research work is under green color small dry pellets form. It is a product marketed in France. It comes from Burkina-Faso.

2.1.3. Ingredients

The dried orange zest and lyophilized orange juice are used as aromatizing and stabilizing ingredients.The powder of the orange zest is obtained by thermal drying at 45°C (drier apparatus, type MELAG 405). Thus, the powder obtained is then conditioned in one limps tinted hermetically closed safe from the air and moisture.

2.2. Analyses methods

They refer to the following experiments:

2.2.1. Physicochemical characterization of the date fruit

The different parameters determined in this research are: the pH (NF V 05-108, 1970); acidity assayable (NF V05-101, 1974); water content (NF V 03-903); content of biogenic salts by spectroscopy of atomic absorption (NF V05-113, 1972); determination of the soluble rates of solids (°Brix) (NF V 05-109,1970); sugars (Nelson, 1944); content of proteins (Method of Kjeldhal (NF V 04-211, 1971; AFNOR, 1999); content polyphenols (Ribereau –Gayon, 1972).

2.2.2. Physicochemical characterization of the Spirulinapowder

The factors taken into account are: the pH; content of proteins; the chemical composition in fatty acids; the separation of the pigments by CCM (Wilmotte, 2007); spectral characterization of the pigments; the quantification of chlorophyll (Ramesh, 2000); the content of phycocyanin was determined by colorimetric by measuring the absorbance with 615nm and 652 nm (Jourdan, 2006). The carotenoid concentration was appreciated by colorimetry; one measures the optical density with 450 nm.

2.2.3. Dates and Spirulina powder microbial characterization

The microbiological analyses of powders of *Mech-Degla*datesand Spirulina are carried out according to the methods recommended by Guiraud (2003). The biological activity of the various

pigments (phycocyanin, xanthophylls, carotenoids and chlorophyll) extracts freeze-dried starting from the powder of Spirulina and of the fresh biomass of Spirulina was given from three stocks (*Aspergillusniger, Pseudomonas aeruginosa*and *Staphylococcus aureus*). This technique makes possible *in vitro*diameter measurements of the inhibition halation (clear zone) (Rahal,2005).

2.2.4. Obtaining the date powder production

In this step oh work, the influence of the couple (temperature / processing time) on the effectiveness of drying was studied. The kinetic studies of drying are necessary to develop suitable mathematical models to lay down the effect of time, temperature, activity of water and other factors on the extent of the changes in a particular food during the drying and storage treatment (Labuza,1973; Nunes and *al.*1991). The selected temperatures are (in °C): 65, 75 and 85.

2.2.5. Obtention and characterization of compressed powder of dates+ Spirulina

2.2.5.1.Choice of the mixture

As a preliminary, seven different formulations were prepared. The choice of the good formulation is based on the sensory analysis (AFC) (BenahmedDjilali and *al.*2011).

2.2.5.2.Compression of the formulations

Tablets having a diameter of 12mm were prepared by direct compression using analternativesemi-automatic equipment (ED FROGERAIS, OA307) at the level of CRD Saidal (El-Harrach /Algiers).

Three compressed formulations (F_1,$F_6$and F_7) were elaborate:

F_1contains 80% powder of dates + 10% powder of Spirulina + 5% powder of dried orange zest + 5% powder of lyophilized orange juice;

F_6contains date powder;

F_7contains powder of Spirulina.

2.2.5.3. Physicochemical characterization of the manufactured tablet**s**

During the manufacture of the tablets, measures are taken in order to obtain tablets having a sufficient mechanical resistance to be able to be handled without exhausting itself, not to break. Preparation of compressed and determination of their physicochemical properties (time of disintegration in distilled water at 37±0,5°C and dissolution in three solutions: (distilled water, HCl 0,1 N and phosphate buffer pH 6,8)were carried out in the laboratory of CRD Saidal (El-Harrach/Alger) according to the European pharmacopeia (EP, 2007)and adapted procedures of the pharmacopeia of the United States by other authors (Sriamornsak and *al*. 2007).

3. Results and discussion

3.1. Results of the characterization of the powders

In this phase of research, the results concerning the physicochemical and microbiological characterization of the dates and Spirulina powders are:1) as regards the Spirulina: it is rich in proteins (61,745 ± 0, 0353; n=3), in essential fatty acids (Fig.1) (C14:O (46,02%), C18: 2 (13,11) C18:3 (9,53%), C18: 2 (13,11 %) and in bioactives substances: phycocyanin (1,7±0,0015g/100g ms; n=3);polyphenols (5,1325%EAG), total chlorophyll (1,0463 ± 0,0946 %; n=3) and of ashes (9,41 ± 0,03%; n=3); 2) as regards date: it is rich especially in sugars (TSS=60 ± 0,4% n=3) but it is low in proteins (2,1 ± 0,4%; n=3) and out of minerals compared to the Spirulina (1,44 ± 0,2%; n=3). Relatively to the biological activity, the sensitivity of three tested microorganismsso as *Pseudomonas aeruginosa, Aspergillusniger*and *Staphylococcusaureus*,with respect totheextract of phycocyanin (C-PC) from the Spirulina was also given. The average diameters of inhibition are 14,10, 10,66 mm for the Spirulina Burkina-Faso, respectively.

Fig.1, illustrates well the profile and the percentages of the components identified by CPG and which are recapitulated in table 1.

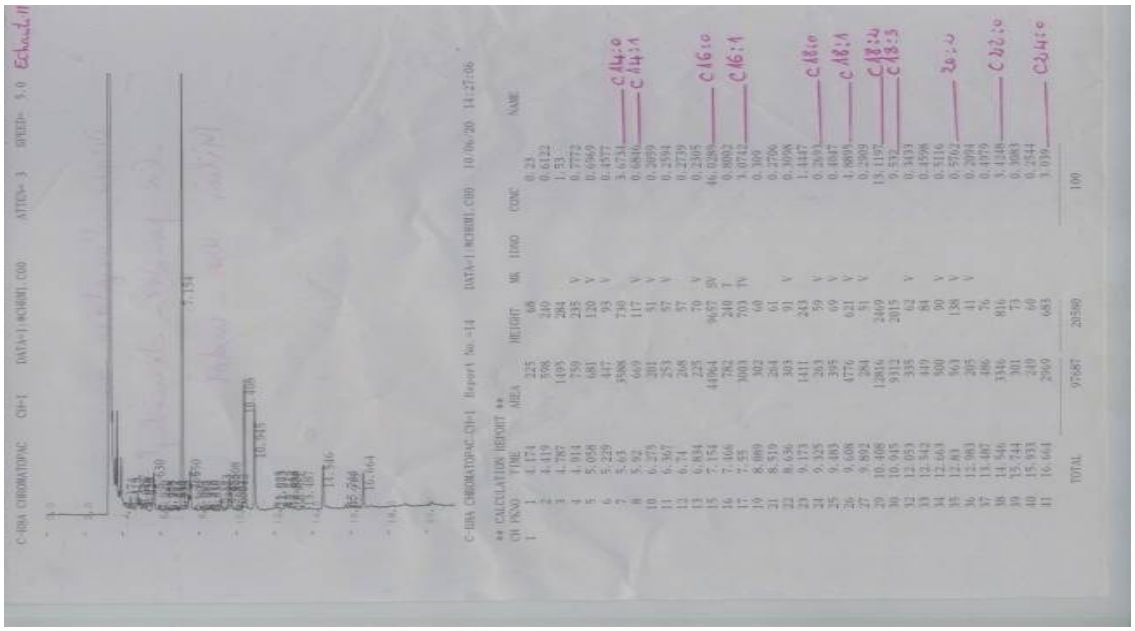

Fig 1:Profile of the fatty acids observed with spirulina from Burkina-Faso.
We present here only the components whose content is higher than 0, 05%.

Table 1: Fatty acids Composition of the Spirulinafrom Burkina-Faso.

Carbonnumbers	Acide fat	Physiological nomenclature	Content (%)	Content (%) (*)
14	Myristique	C14 :0	3,67	(0,2-0,5)
16	Palmitic	C16 :0	46,02	(25-60)
16	Palmitoléique (Oméga 6)	C16 :1	3,0742	(0,5-10)
18	Stéaric	C18 :0	0,26	(0,5-2)
18	Oléic (Oméga 6)	C18 :1	4,89	(0,4-16,6)
18	Linoléic (Oméga 6)	C18 :2	13,11	(10-30)
18	γ- Linolénic (Oméga 6)	C18 :3	9,53	(8-40)
22	Béhénique	C22 :0	3,42	-

(*): According to liu and Liang (1999); Flaquet and Hunri (2006).

The two powders are revealed, under the experimental conditions having a good microbiological quality.

3.2. Some chemical physico–characteristics of the tablets

The factorial analysis of correspondences (AFC)showed that the formulation F_1(80% powder of dates+10% powder of Spirulina+5% powder of dried orange zest+5% powder of lyophilized orange juice;is appreciated,which is translated by a strong density of tasters compared to F_6 and F_7 and this according to the graph Biplotof Gabriel (Fig. 2). It is noticed that Odor parameter is considered and F_6it proves to be the best compared to the other groups of products (richer in Spirulina) which are located all with dimensions negative axis " F1 "(Fig.3).

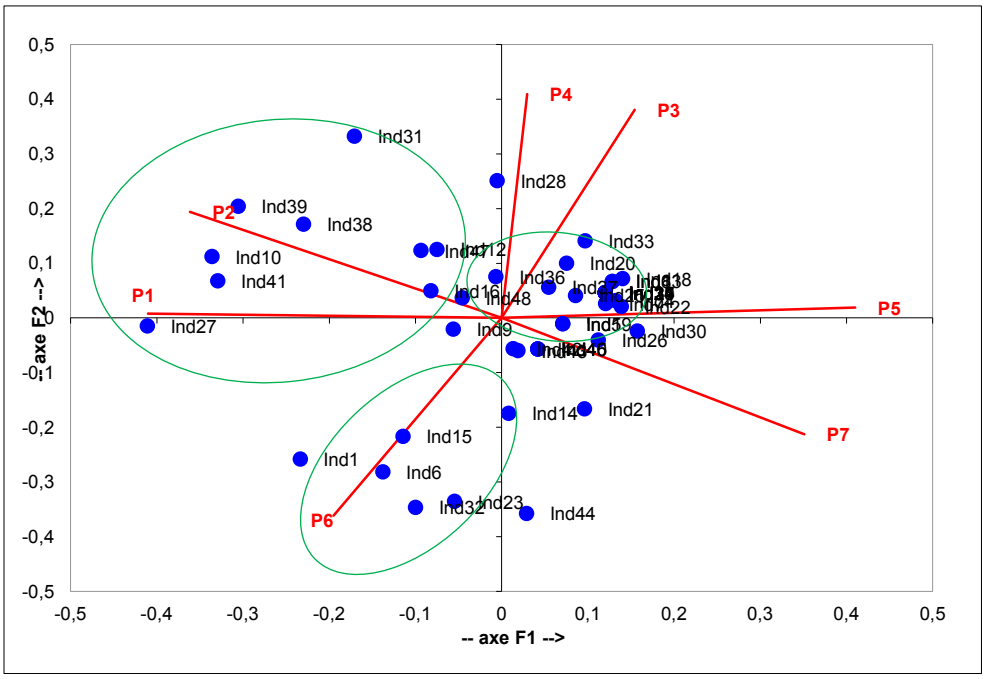

Fig 2: Biplot of Gabriel (axes F1and F2) (appearance criterion).

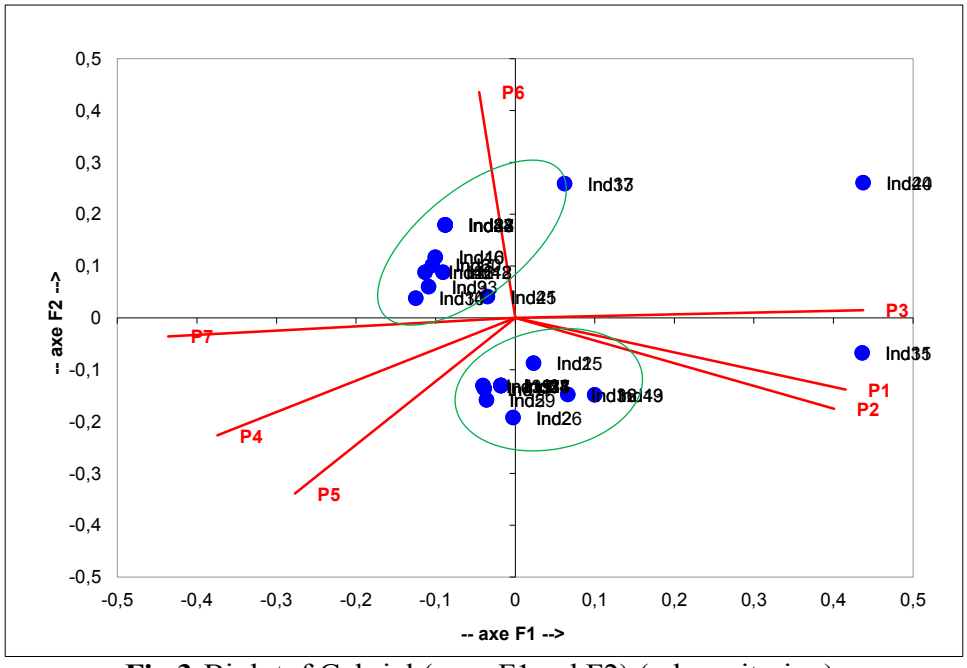

Fig 3: Biplotof Gabriel (axes F1and F2) (odor criterion).

According to the preliminary mechanical characterization, the force 10 T proved to be optimal to obtain tablets with the acceptable friability when below 10 T, the tablets are very friable and above this value, the tablets are excessively hard. In fact, it is well-known that the compressive force represents the principal factor which determines the rate of erosion. The

Spirulinapure powder has a time of flow (flow) high (60 S). What can be due to its hygroscopicity. This last property is responsible for the agglomeration and compressing during storage, therefore induces an adverse effect from the point of view compaction (Emami and Tabil, 2007). Indeed, this disadvantage became as favors of which formation of compressed products (Bimbenet, and *al*. 2002).

3.3. Test of disintegration

The test of disintegration is generally adopted to control the amount of minerals of the vitamins released in distilled water with 37°C (Löbenberg and Steinke, 2006). The time of disintegration,examined for our tablets immersed in distilled water, proved in agreement with those found by certain authors about compressed having various compositions of plants: 6–12 minutes (tablets of *ilicifolia of Maytenus*(Soares and *al*.2005) and 0,31–11,28 minutes (tablets of the paracetamol) (Lefevre and Deceived, 2003). The tablets corresponding to the formulation F_7(pure Spirulina) have a texture lasts and undergo a continual hydrant without erosion while the two other formulations ($F_1$and F_6) disaggregate quickly in all the liquid media applied. This is confirmed by the morphological examination of compressed during the immersion in distilled water (A), HCl 0,1N (b) and phosphatebuffer pH 6,8 (c) (Fig 4).

A B C

Fig 4: Morphological aspect of compressed F_1, F_6 and F_7 during the immersion in distilled water (A), HCl 0,1N (B) and phosphate buffer pH 6,8 (C).

The composition of compressed form can influence the phenomenon of erosion considerably. After 60 minutes of immersion in the three mediums, the rates of erosion can be deduced as follows: 1) distilled water: > 90% (F_1 , 80 –90% (F_6 and < 30% (F_7 ; 2) HCl 0,1.N: > 90% (F_1 , ~70% (F_6 and < 20% (F_7 ; 3) phosphate buffer pH 6,8: ~70% (F_1 , ~90% (F_6 and < 30% (F_7).

3.4. Dissolution of the phycocyanin

The process of dissolution is naturally influenced by the capacities of compressed from with erosion and disintegration. The rate of dissolution of the phycocyanin of compressed formulation F_1increases considerably in distilled water compared to both others liquidate examined. What could be due to the molecular structure of the substance?But, the low rate of release (16% in 45 minutes) of the phycocyanin is a significant factor in terms of its therapeutic properties since low dose of phycocyanin is more effective like anti-inflammatory drug substance. Besides, this result

is in agreement with the investigations about various solutions of extraction of the phycocyanin (JayantMahadev, 2005). This author observed that the highest concentration was obtained with the phosphate buffer pH 6,8 while HCl 0,1N leads toa rate of negligible diffusion of phycocyanin. Moreover, several studies showed % of release of 1 H with 30% for the paracetamol (Parojcic and *al.*2008) and of 12% for the metronidazole (Limmatvapirat and *al*.2008). This behavior makes the tablets more interesting for the cast iron in the mouth (neutral pH). So, the equation of Korsmeyer–Peppas suitably shows the experimental data of solids with R^2 = 0,846 for all the mediums of immersion used. The diffusion of phycocyanin is considered asFickiantype in distilled water and/or HCl 0,1N whereas dissolution in the phosphate buffer at pH 6,8 becomes of non-Fickian type. Our results are in agreement with those found with certain drugs incorporated in various tablets formulated using polymeric absorbent, alginates and variouspectins(Korsmeyer, 1983;Sriamornsak, 2007).

4. General conclusion

Taking into account the interesting results obtained, it appears of a great utility to continue the present study while touching, in the future, with various axes with an aim of looking further into scientific and technological knowledge to contribute to draw more information and information putting at light the food and medical role of the exotic and/or natural products. For this, our search team plan to undertake the various important research activities as the glycemic index and nutraceutic effects of the formulation F_1. We also have a goal concerning the optimization ofSpirulina culture and its waste products valorization and also the creation of a new activity in crino Food with various activities (plantation and palm protection, production, processing, packaging, marketing and health impact).

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