# PHYTOCHEMICALS CONTENT, ICP-OES METALS DETERMINATION AND PROTECTIVE EFFECT OF CONSUMPTION OF DATES ROBE ON ANEMIA DISEASE RISK IN HEALTHY WOMEN

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*(Received 09 February 2022- Accepted 13 May 2022)*

***Abstract.-*** *The robe is made from the date palm fruit for the use in nutritional and medicinal fields. The main aim of this investigation was to identify the phytochemical and mineral contents in two varieties of date "Robe" Ghars and Tekrmist (Tantbocht) of Algerian* Phoenix dactylifera *L. and to evaluate the effect of consumption of dates robe on anemia disease risk in healthy women. The amount of all metals was determined by ICP-OES technique. Qualitative and quantitative analysis of phytochemical compounds were estimated by using standard protocols. Two groups of 20 volunteers healthy women in each one were chosen to eat 8g of robe Ghars and Tekrmist (Robe) twice a day during 15 days. Some biochemical and hematological parameters were measured. The qualitative and quantitative results of phytochemical analysis revealed that Robe of Ghars and Tekrmist rich by reducing compounds, flavonoids, terpenoids, saponins and tannins, however they are characterized by the absence of alkaloids, in addition the both dates robe contain an important quantity of total sugar. Results indicated that Robe of the both varieties was considered as the principal source of essential elements (Ca, Na, K, Mg, Fe, Zn, Cu, Mn). The consumption of dates robe Ghars and Tekrmist during 15 days led to significant increase in serum folate, cobalamin and ferritin, and no significant change in the other parameters. We concluded that dates robe " Robe" of Ghars and Tekrmist varieties of* Phoenix dactylifera *L. contains important nutritive and bioactive compounds may have significant potential for to protecting against various diseases including anemia disease.*

***Key words****: Robe; Ghars; Tekrmist; phytochemical compounds; ICP-OES technique; Anemia.*

# CONTENU PHYTOCHIMIQUES, DETERMINATION DES METAUX PAR ICP-OES ET EFFET PROTECTEUR DE LA CONSOMMATION DE ROBE DE DATTES SUR LE RISQUE D'ANEMIE CHEZ DES FEMMES SAINES

***Résumé****.- Le robe est fabriquée à partir des dattes pour être utilisé dans le domaine nutritionnel et médicinale. L'objectif de cette étude était d'identifier la teneur phytochimique et minérale de deux variétés Algériennes de Robe de dattes (*Phoenix dactylifera *L) Ghars et Tekrmist (Tantbocht) et d'évaluer l'effet protecteur de la consommation de robe de dattes sur le risque d'anémie chez des femmes sains. La teneur en métaux a été déterminée par la technique ICP-OES. L'analyse qualitative et quantitative des composés phytochimiques a été estimée en utilisant des protocoles standards. Deux groupes de femmes volontaires sains dans chacun 20 femmes ont été choisis pour consommer 8g de robe de Ghars et Tekrmist deux fois par jour pendant 15 jours. Certains paramètres biochimiques et hématologiques ont été mesurés. Les résultats de l'analyse phytochimique qualitatives et quantitatives ont révélé que la Robe de Ghars et de Tekrmist sont riche en composés réducteurs, flavonoïdes, terpénoïdes, saponines et tanins, avec l'absence d'alcaloïdes, de plus les deux robe de dattes contiennent une quantité importante de sucre totaux. Les résultats ont indiqué que le robe des deux variétés était considérée comme importantes source des éléments essentiels (Ca, Na, K, Mg, Fe, Zn, Cu, Mn). La consommation de robe de dattes Ghars et Tekrmist pendant 15 jours a entraîné une augmentation significative du folate, du cobalamine et de la ferritine sériques, et aucun changement significatif des autres paramètres. Nous avons conclu que le robe de dattes (*Phoenix dactylifera *L.) de variétés Ghars et Tekrmist*

*contient d'importants composés nutritifs et bioactifs pouvant avoir un potentiel protection contre diverses maladies, y compris l'anémie chez les femmes.*

***Mots clés** : Robe; Ghars; Tekrmiste; composés phytochimiques; technique ICP-OES; anémie.*

## Introduction

To obtain food and medicine, the human societies have used components of the environment through their strong contact with the environment, since the beginning of their formation [1]. Many of recent landmarks in scientific research have shown that in human beings, oxidative stress is an important factor causing metabolic and physiological alterations and various diseases in the body [2]. Phytotherapy science uses the medicinal plants and herbal products for prevention and treatment of different diseases [3]. the date palm (*Phoenix dactylifera* L.) is among the most important desert flora. Dates support the health of desert-oasis ecosystems as well as having important nutritional, economic, social, and historical values [4]. The date palm is one of the most available fruits in the world [5]. Since old times, its crop was important in the arid and semiarid regions [6]. Date fruits include many nutrients such dietary fiber, sugar and other minerals (phosphorus, calcium, potassium…) which makes the dates nutritionally more important for humans [7]. The date palm is also a multi-purpose plant [8]. It has a therapeutic and nutritional importance with significant anti-proliferative, antioxidant, antifungal and antibacterial properties [9]. These effects have been demonstrated to result from various active components, including alkaloids, fatty acids, flavonoids, polysaccharides, and terpenoids [10]. There are many date products, including date robe [11], which is used locally in many nutritional and therapeutic uses. The objective of this study is the characterization of phytochemical and minerals in dates “Robe” of two varieties Ghars and Tekrmist of Phoenix dactylifera L., also, to affirm the traditional use of robe to prevent anemia disease based on its phytochemical composition.

## 1.- Materıal and methods

### 1.1.- Chemicals and reagents

All of the chemicals and reagents were analytical grade and provided from Merck, 134 (Darmstadt, Germany). In this research study, we employed deionized water and nitric acid 135 (65%), with spectroscopic grade, given by Merck (Darmstadt, Germany).

### 1.2.- Preparation of Robe

The fruits that we used in the preparation of the robe are the Ghars and the Tekrmist, which we picked at the end of the ripening stage from the El-Oued region, southeast of Algeria. The robe was prepared from the dates of Ghars and Tekrmist by taking 50g of washed dates and cooking with 100ml of water within 30 minutes at 70°C. Then, this mixture was filtered with a Josef paper after letting it cool to get the juice which simmered at 100°C for 2 hours until obtaining the robe of dates.

### 1.3.- Phytochemical analysis

The phytochemical screening of Robe for identifying the phytochemical compounds present in it was made according to stander protocols [12]. The estimation of total phenol

was determined by the Folin-Ciocalteu method [13]. The method described by Ahn et al. was used for the determination of the total flavonoid content [14]. Determination of sugars and related substances were performed a colorimetric method as described by DUBOİS *et al*. (1956) [15].

### 1.4.- Metals quantification method

#### 1.4.1.- ICP-OES method

The optical emission spectrometer SPECTRO ARCOS (SPECTRO Analytical Instruments, Kleve, Germany) with axial plasma observation was employed for the metallic elements measurements The instrument features a spectrometer mount Paschen-Runge, whose working principle is based on the Rowland perfect circuit alignment (ORCA) technique. The device consists of two hollow sections, an optimized compact size, and 32 linear charge coupled device detectors (CCDs), the wavelength range between 130 and 770 nm which can be measured together at the same time, allowing the capture of complete spectrum within 2 seconds.. Due to unique system of reprocessing capabilities, it's necessary to not require new measurement even if additional lines or elements are to be identified at a later date. The optic is closed hermetically and loaded with argon and continuously circulated by a filter, which absorbs vapor, water, oxygen and other species. In the vacuum ultraviolet (VUV), high optical transmission is achieved, allowing the identification of nonmetals as well as the use of distinguished and interferences-free lines in this region. Employing Intelligent Calibration Logic (ICAL), which regulate the wavelength scale, the situation of the optical system is controlled automatically.

#### 1.4.2.- Calibration standards

For calibration, commercially available standard solutions were used. The quality control of the method was performed using NIST standard reference material (SRM) 1640, "Trace Elements in Natural Water." All solutions were prepared with nitric acid, suprapure quality.

### 1.5.- Study design

A sample of 20 volunteer women aged 20 to 24 years old, residing in the El Oued region located in the southeast of Algeria was randomly distributed equally between two groups (fig. 1). Each group consumed 8g (4 g every 12 hours) of either the Gars robe dates or Tekermist robe dates every day (half an hour before any meal) during the 2-week study period.

Contact information was received for 28 women initially interested in participating in the study. After we lost contact with each other (2 cases) and others not interested to participate ( 6 women), the number reached 20 women who were enrolled in the study. To eliminate the factors which might affect biochemical and hematological parameters, we excluded all diabetics, anemia and other chronic diseases subjects.

For each volunteer in this study, we recorded his information related to age, height, weight and blood pressure. We also calculated the Body Mass index (BMI) according to the following method: BMI= weight (kg)/ (height m)$^2$.

The research protocol was approved by the department of cellular and molecular biology, El Oued University Ethics Committee (approval number: 23 EC/DCMB/FNSL/EU2021).

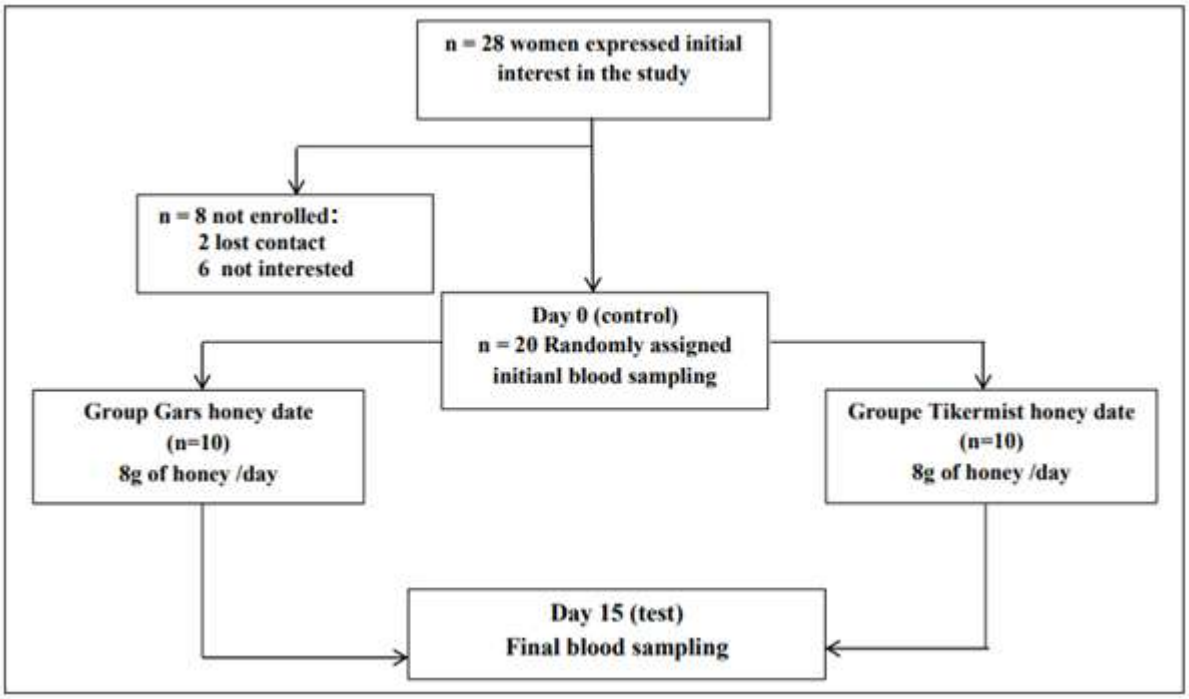


**Figure 1.-** Flowchart of subject recruitment, enrollment, and retention from women

### 1.6.- Samples collection, Blood biochemical and hematological analysis

Fasting blood samples were collected and placed into EDTA and heparin containing tubes. Blood was transferred into EDTA tubes for hematological studies and the serum were obtained after centrifugation of blood in heparin tubes at 3000 × g for 5 min, removed and retained for assay of the level of glucose and biochemical parameters.

Hematological analysis (FNS) was performed by the hematology autoanalyzer (Abacus 380). Serum electrolyte levels ($Fe^{3+}$ and $Ca^{2+}$) and blood glucose, uric acid concentrations were measured using commercial kits (Biomagreb, Tunisia). Ferritin, folate and cobalamin were measured by the autoanalyzer (Maglumi 1000) and using commercial kits (Spinreact Lab).

### 1.7.- Statistical analysis

The reported data are the means of measurements and their standard error of mean (SEM) values. The results of cases were compared by student test using MINITAB software version 13, differences were considered statistically significant at the 5% level.

## 2.- Results and discussion

### 2.1.- Clinical characteristic of volunteers

The general data characteristics of the two groups of subjects include age, Body Weight, Body Mass index (BMI) and blood pressure levels. These indicators do not have any statistically significant ($P > 0.05$) in two group studies as shown in table I.

**Table I. -** Characteristic of volunteers

| Parameters | Robe Ghars (n=10) | Robe Tekrmist (n=10) | P-value |
|---|---|---|---|
| **Age (ys)** | 23.10±1.47 | 21.11±0.84 | 0.182 |
| **Body Weight (kg)** | 63.06±3.63 | 66.48±3.52 | 0.092 |
| **Body Mass index** | 25.32±0.38 | 25.33±0.43 | 0.252 |
| **Systolic blood pressure (mmHg)** | 108.85±2.7 | 109.68±3.4 | 0.637 |
| **Diastolic blood pressure (mmHg)** | 68.81±4.7 | 78.42±2.6 | 0.053 |

## 2.2.- Qualitative and quantitative phytochemical analysis

In this study, we detected the presence of various phytochemical compounds (flavonoids, terpenoids, reducing sugar compounds and saponins) in the both types of robe dates studied as presented in table II. However, these two types of robe poor in alkaloids. The tannins are present only in robe prepared from Ghars date. These results were probably similar to that find by GAYATHRİ and THİLAGAVATHİ (2021) [16], who informed that two unripe Indian varieties of dates of Phoenix dactylifera namely Barhi and Khenaizi possess the different phytocompounds specially in aqueous and methanolic extracts. ECHEGARAY *et al.* (2021) [17], demonstrated the potential healthy proprieties of date fruit through their richness in bioactive compounds which make that appropriate to use as essential nutraceutical ingredients in several foodstuffs.

**Table II.-** Phytochemical analysis of robe dates Ghars and Tekrmist
(+: Presence, -: Absence)

| Phytochemical | Robe (Ghars) | Robe (Tekrmist) |
|---|---|---|
| **Flavonoids** | + | ++ |
| **Tannins** | +++ | - |
| **Alkaloids** | - | - |
| **Terpenoids** | + | ++ |
| **Saponins** | ++ | ++ |
| **Reducing sugar compounds** | + | ++ |

The results of quantitative phytochemical analysis were presented in table III. The total phenol contents in dates robe Ghars and Tekrmist vary from 206 to 281 mg EGA/100g of Robe, this range may be considered include in the range from 209 to 448 mg GAE/100 g of fresh weight in date cultivars consumed in Tunisia [18]. The total flavonoid contents in robe Ghars and Tekrmist ranged between 215 and 258 mg EQer/ 100g of Robe, these results confirmed by study of ALAHYANE *et al*. (2019) [19], 2019 which demonstrated that content of total flavonoids in seventeen Moroccan varieties and clones of dates varies between 1.79 and 216.12 mg EQer/ 100 g of dry material. The robe Ghars and Tekrmist are a considerable source of bioactive molecules which can contribute to the antioxidant potential, provide health promoting, disease preventing effects and can reduce the risk of diseases due to oxidative stress [20,21]. The quantification of total sugar demonstrated that two varieties were very rich in sugar more than 4620 mg EGlu/100g of dry material as presented in a previous study of Assirey [22], who informed that total sugar in 10 of date palm fruit cultivars grown in Saudi Arabia changed between 71.2-81.4 mg EGlu / 100 g of dry material.

**Table III. -** Total phenol, flavonoid and total sugars contents in dates robe (Robe) Gars and Tekrmist

| Samples | Total phenols (mg EGA/ 100 g) | Flavonoids (mg EQer/ 100 g) | Total Sugars (mg EGlu/100g) |
|---|---|---|---|
| **Robe (Ghars)** | 206±20.2 | 215±4.38 | 4621 ± 57.13 |
| **Robe (Tekrmist)** | 281±4.15 | 258±15.06 | 4675±111.6 |

## 2.3.- Meniral contents

Table IV showed the essential elements present in robe Ghars and Tekermist and detected by ICP-OEM analysis with different concentrations, when the majors were calcium, sodium, potassium and magnesium, while the trace elements were iron, zinc, copper, manganese, cobalt, chrome, lithium, bore and bismuth, Where we found that the quantities of both calcium and sodium are greater in robe Ghars compared to Tekermist, while we found the opposite for potassium and magnesium. Also, robe Ghars contains much larger quantities of essential trace-elements than robe Tekermist, as well as for most of the non-essential trace elements. FARİD *et al*. (2020) [23] reported that most of the previously mentioned elements such as calcium, magnesium, copper, zinc, manganese, iron, sodium and potassium were detected in Egyptian Phoenix dactylifera fruits. Potassium and phosphorous are impart strength for human cell regeneration, while magnesium and calcium are essential for healthy development of bones, also the Iron is very important for the production of blood and tissue respiration [24]. In general, the presence of these essential elements, robe Ghars and Tikrmest may be considered as healthy diet and rich source of essential minerals and play a critical role in the development of the human immune system.

**Table IV.-**Concentration of major, essential and non-essential elements in robe Ghars and Tekermist

| | Elements | Robe Ghars | Robe Tekrmist |
|---|---|---|---|
| **Major elements (µg/g)** | Calcium | 73.9±0.2 | 2.8±0.05 |
| | Sodium | 55.01±0.23 | 0.6±0.006 |
| | Potassium | 0.12±0.003 | 4.3±0.06 |
| | Magnesium | 0.27±0.001 | 4.01±0.007 |
| **Essentials trace elements (ng/g)** | Iron | 976.5±19.2 | 223.6±4.36 |
| | Zinc | 530±19.4 | 173.2±19.19 |
| | Copper | 96.51±11.4 | 9.51±0.91 |
| | Manganese | 201.7±12.2 | 0.447±0.0035 |
| | Cobalt | 2.79±0.14 | 0.36±0.0025 |
| | Chrome | 17.41±1.21 | 4.67±0.57 |
| | Lithium | 130.8±20.7 | 0.38±0.005 |
| | Bore | 523.8±22.7 | 2.93±0.024 |
| | Bismuth | 402±2.83 | 0.31±0.0024 |
| **Non essentials trace elements (ng/g)** | Baryum | 41.73±1.2 | 0.339±0.0035 |
| | Aluminium | 95.4±3.57 | 16.47±0.54 |
| | Strontium | 2.05±0.21 | 0.912±0.002 |
| | Lead | 134.9±12.5 | 84.39±7.002 |

| | | |
|---|---|---|
| Cadmium | 2.055±0.12 | 5.46±0.006 |
| Gallium | 3.66±0.12 | 2.37±0.0054 |
| Silver | 2.51+0.01 | 0.47±0.07 |
| Indium | 18.17±1.7 | 4.11±0.29 |
| Nickel | 13.2±0.01 | 9.45±0.08 |

## 2.4.- Biochemical parameters

Table V presented the consumption effect of robe dates of Ghars and Tekrmist on levels of biochemical biomarkers, when we observed a significant increase in levels of serum calcium, ferritin, folic acid and cobalamin, were observed after 15 days of consumption of robe Ghars. However, the consumption of robe Tekrmist leads to a significant increase in uric acid levels after 15 days. The effect of both Ghars and Tekrmist to lower the level of blood glucose may be related to anti-diabetic impact of bioactive compounds such as flavonoids, saponins and phenol present in *Phoenix dactylifera* extract, this in agreement with study of MIMOUNI *et al*. (2022) [25] which indicated that the date syrup makes it possible to classify it among the products with a low glycemic index likely to be intended for diabetics and the obese. while, the mechanism of action of dates in the control of diabetes is not fully understood, but it could be due to the increase of insulin production and inhibition of glucose absorption [26]. A significant increase in serum calcium level can be interrupted by the presence of high amount of calcium element in robe Ghars 73.9±0.2 µg/g of Robe which confirmed by the ICP-OEM analysis. also another study of MOHAMMADZAİ *et al*. (2010) [27], who found a high amount of calcium were up to 84.40±0.9 µg/g of date palm fruit available in Pakistan. The variation in levels of folic acid and cobalamin in the serum after consumption of each of robe Ghars and Tekrmist may be reflected to their containing on both vitamins as what reported by Aslam et al. [28], that quantified water soluble vitamins in dates may be varied between the cultivars also between their developing stages. ABDEEN (2018) [29], informed that vitamins B2, B9, B12 were detected in immature dates inverse to vitamins B1, B3, B5, B6 which were determined in mature stages with high amount. The synthesis of ferritin is mainly regulated by the iron level [30], this last is present in the robe Ghars (976.5±19.2 ng/g) which detected by ICP-OEM analysis, also other study confirmed the containing of iron in the date palm fruits (*Phoenix dactylifera* L.) [31]. The antioxidant activity of phenolics and flavonoids compounds present in almost of dates palm fruit varieties plays an important role in scavenging free radicals [32] what led to rise in serum uric acid level and made it unused despite being a major antioxidant in the human plasma [33]. According to the ANOVA test, we observed a non-significant change between consumption of each two varieties Ghars and Tekrmist in the all parameters with exception the ferritin level which confirm the convergence of their therapeutic effect in the body (P =0.038).

**Table V. -** Effect of consumption of robe Ghars and Tekrmist on biochemical parameters in healthy women

| | **Robe Ghars** | | **Robe Tekrmist** | | **ANOVA Significant** | |
|---|---|---|---|---|---|---|
| | **Control (Day 0)** | **Test (Day 15th)** | **Control (Day 0)** | **Test (Day 15th)** | **Day 0** | **Day 15th** |
| **Blood glucose (g/l)** | 0.87 ± 0.029 | 0.83 ± 0.01[a] | 0,90 ± 0,01 | 0,82 ± 0,02[c] | P > 0.05 | P > 0.05 |
| **Serum calcium (mg/l)** | 89.21 ± 2.43 | 94.19 ± 2.12[b] | 94.62 ± 1.71 | 90.03 ± 1.76[c] | P > 0.05 | P > 0.05 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Uric acid (mg/l)** | 34.48 ± 2.34 | $35.84 \pm 1.53_{NS}$ | 31.54 ± 1.42 | $36.07 \pm 1.08^{a}$ | P > 0.05 | P > 0.05 |
| **Ferritin (ng/l)** | 11.78 ± 1.03 | $21.93 \pm 4.63^{a}$ | 13.43 ± 1.28 | $15.06 \pm 1.90^{NS}$ | P > 0.05 | P < 0.05 |
| **Folic acid B9 (ng/l)** | 13.32 ± 0.51 | $14.84 \pm 0.56^{a}$ | 12.76 ± 2.70 | $12.53 \pm 1.79_{NS}$ | P > 0.05 | P > 0.05 |
| **Cobalamin B12 (pg/l)** | 262.7 ± 12.60 | $352.1 \pm 22.1^{b}$ | 243.7 ± 69.3 | $355.8 \pm 14.2^{c}$ | P > 0.05 | P > 0.05 |

Letters at the top of the values in the same row represent significant differences between each parameter determined in test women compared to the corresponding one in control women ($p < 0.05$). Differences were tested using student test.

## 2.5.- Hematological parameters

A decrease in RBC and hemoglobin necessarily leads to anemia disease [34] but from table VI, the consumption of dates robe of the two varieties Ghars and Tekrmist did not lead to significant variation the hematological parameters, while the robe Ghars gave a significant decrease on the number of white blood cells (WBC) and lymphocyte (LYM) in healthy women during 15 days, this effect may have linked to immunomodulatory and anti-inflammatory impacts of date palm fruit (*P. dactylifera*) [35]. Another explanation may be responsible for these effects is a richness of Ghars in bioactive substances such as tannins, flavonoids, terpenes and sugars, as demonstrated in the phytochemical analysis, which possess various biological properties related to antioxidant and anti-inflammatory mechanisms by targeting reactive oxygen species [36]. According to the ANOVA test, we observed a non-significant change between consumption of each of the two varieties Ghars and Tekrmist in the all parameters with exception the immunological lines which confirm the convergence of their therapeutic effect in the body (P 0.013 For WBC and P =0.006 For LYM).

**Table VI. -** Effect of consumption of robe Ghars and Tekrmist on hematological parameters in healthy women

| | **Robe Ghars** | | **Robe Tekrmist** | | **ANOVA Significant** | |
|---|---|---|---|---|---|---|
| | **Control (Day 0)** | **Test (Day 15th)** | **Control (Day 0)** | **Test (Day 15th)** | **Day 0** | **Day 15th** |
| **WBC ($x10^9$/l)** | 6,74 ± 2,40 | $4,90 \pm 1,07^{c}$ | 5,86 ±2,10 | $6,127 \pm 2,32^{NS}$ | P > 0.05 | P < 0.05 |
| **LYM ($10^9$/l)** | 2,16 ± 0,66 | $1,796 \pm 0,36^{c}$ | 2,12 ± 0,51 | $2,26 \pm 0,50^{NS}$ | P > 0.05 | P < 0.05 |
| **RBC ($x10^{12}$/l)** | 4,63 ± 0,65 | $4,578 \pm 0,33^{NS}$ | 4,57 ± 0,32 | $4,21 \pm 0,90^{NS}$ | P > 0.05 | P > 0.05 |
| **HB (g/dl)** | 12,39 ± 1,25 | $12,10 \pm 2,98^{NS}$ | 12,45 ± 1,58 | $11,16 \pm 3,23^{NS}$ | P > 0.05 | P > 0.05 |
| **HT (%)** | 37,96 ± 3,69 | $38,95 \pm 2,92^{NS}$ | 38,54 ± 3,91 | $34,45 \pm 9,38^{NS}$ | P > 0.05 | P > 0.05 |
| **PLT ($x10^9$/l)** | 260,2 ± 98,0 | $252,6 \pm 81^{NS}$ | 258,20 ± 40,29 | $243,3 \pm 43,81^{NS}$ | P > 0.05 | P > 0.05 |

Letters at the top of the values in the same row represent significant differences between each parameter determined in test women compared to the corresponding one in control women ($p < 0.05$). Differences were tested using student test.

## Conclusıon

We conclude that dates robe " Robe" of both varieties Ghars and Tekrmist (Tantbocht) of *Phoenix dactylifera* L. are rich in different bioactive phyto-compounds (flavonoids, terpenoids, reducing sugar compounds and saponins) and essential mineral

elements (calcium, sodium, potassium, magnesium, iron, zinc, copper, manganese, cobalt and chrome) which give it a nutritional and medicinal importance through their beneficial effects on the various hematological and biochemical parameters in healthy women.

**Acknowledgements:** The authors thank the members of the Atomic Spectrophotometers Laboratory, Faculty of Chemistry, and UiTM University- Malaysia for providing research facilities to carry out present work. This work was supported by the research project D01N01UN390120190001 funded by the ministry of higher education, Algeria and by Directorate general for Scientific Research and Technological Development.

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