Nutritional Evaluation, Antioxidant, Anticancer and Antimicrobial Activities of Egyptian amhat date palm fruit

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Abstract

Amhat date (soft variety) is one of popular fruit in Egypt. The varieties in Egypt were soft, semi dry and dry, most of Egypt's production of dates is soft, amhat date variety in Egypt is consumed in rutab stage. Therefore, it was necessary to be ripened artificially to increase the shelf life and prevent its unacceptable taste. So, the chemical composition of amhat date were estimated (moisture, total soluble solids, crude protein, fats, crude fiber) . Also , its minerals content were estimated , and the nutritional values of amhat date palm (khalal , natural rutab and artificial rutab) were measured .The bioactive compounds were estimated (total phenolic compounds , simple phenols , total tannins, hydrolysable tannins and condensed tannins) fractionated phenolic compounds and flavonoids by HPLC (High Performance Liquid Chromatography)were used for measuring the responsible phenolic compounds of anticancer (caffeine , p-coumaric acid and ferulic acid) So, the bioactine compounds have important role of khalal amhat date , natural rutab and artificial rutab amhat date as antioxidant , anticancer and antimicrobial agents.

Key words: Amhat date – Chemical composition- phenolic compounds – flavonoid compound – anticancer activity – antimicrobial activity.

Introduction

The total world production of dates is 8460443 tons and Egypt produced 1694813 tons of dates (**FAO, 2016**). Egyptian dates represent more than 70 % soft dates and one of important soft date is Amhat date variety which represent 29193 tons (**A.A.C.S. 2016**).

The chemical analysis of this variety in khalal stage was 70.0% for its moisture content and 4.5 for pH value and the total acidity ,reducing sugar, total sugar and total free phenolic compounds were 0.56 , 35.06, 66.4 and 3.00 % (on DW) of Amhat date of khalal stage. While the rutab stage content 50.05% moisture and pH value was 5.5 also contained 0.042 , 70.50 , 71.30 ,2.12 and 1.10 % of total acidity , reducing sugar ,total sugar , total free amino acid and total free phenolic compound respectively . as reported by **(Nezam El.Din and Abd EL-Hameed,2003).**

Abo Taleb *et al.* , (2018) reported that the chemical composition of rutab amhat date was as follows: the moisture was 99.73% , protein was 1.5% , fat 1.3% , total soluble solid 28.65% , PH value 6.76% , total acidity was 0.190% and Ash content was 1.84% (on DW)**.** And they observed that Amhat dates had the highest amount of fructose glucose , mannose and galactose (41.45, 32.32, 17.68, 17.03%) respectively. But had the least amount of sucrose was 0.189% by using HPLC for sugar analysis**.** Also, they found that the mineral content of fresh amhat dates were Potassium (K) was 370.12mg/100g, Magnesium (Mg) was 91.756 mg/100g , calcium (Ca) was 157.56 mg/100g , Magnesium (Mg) was 0.70 mg/100g , Iron (Fe) was 0.721 mg/100g and zinc (Zn) was 0.309 mg/100g.

The bioactive compounds which are found in some food played good roles as antioxidant, anticancer and antimicrobial, Some of these compounds are phenolic compounds, flavonoids ,tannins, anthocyanidin (**Seeram *et al*.,2005 and Du *et al*.,2012**). Previous components are found in some popular Egyptian food such as date palm fruit .

Date palm fruit is rich by tannins and phenolic compounds especially green and yellow of date fruit (*Pheonix dactylifera L.*) . The previous compounds showed clear changes during ripening especially in soft dates from khalal to Rutab stages (**Nezam El-Din and Abd El- Hameed.,2003**) .

Abo Taleb *et al*., (2018) fractionated the amount of individual phenolic acids present of fresh Amhat date by HPLC. And they found that Gallic acid was 5.872 mg/100g Ellagic acid was 6.075 mg/100g and the highest amount was e-vinillic acid 50.68 mg/100g on dry weight basis. And they also found the quantities of flavonoids compounds were fractionated by HPLC. Were Rutin was 4.382 mg/100g , Naringin was 1.247 , Quercitrin was 1.991 mg/100g , Quercetin was 0.509 mg/100g and the highest amount was Acacetin 10.82 mg/100g**.**

Benmeddour *et al*., (2012) studied that the identification and quantification of flavonoids compounds by HPLC (High Performance Liquid Chromatography) and observed that the iso quercetin was 3.03 mg/100g DW in Ghazi varity and rutin was 2.78 mg/100g DW in Sebt Mira varity .

The tannin content of different in UAE date palm fruit (Bushibal, Gash Gaafar, Gash Habash, lulu and

shahla) in different stage (khalal and Rutab) were 1.3, 0.9, 1.4, 0.9, 1.6, 1.2, 1.2, 0.8, 1.3% DW) respectively as observed by **Al-Hooti *et al*., (1997).**

Abo Taleb *et al*., (2018) found that the antioxidant activity of amhat dates was 63.3% (on dry weight basis).

The methanolic extract of Ajwa date inhibit human breast Adenocarcinoma (MCF7) they found that a decrease in cell number at 20 mg/ml concentration for 48 hour and 72 h. were ranged from 78.20% to 96% respectively and at 25 mg/ml concentration for 72 h was 99.12% as reported by **Khan *et al*.,(2016).**

Saleh and Otaibi (2013) observed that ethanolic extract of three date palm varieties (Sheshi, khulase and Rezaz). And they found that in Biser stage had the strongest Antimicrobial activity by Staphylococcus saprophyticus (the diameter of inhibition zone 30, 35 and 37.5 mm) respectively.

So, this study aims to evaluate the nutritive values date fruit (amhat variety) at Khalal stage and Rutab stage. Studing the effect of this food as antioxidant, anticancer and antimicrobial agents**.**

Material and Methods

Materials

Amhat dates variety (*phoenix dactylifera L.*) at Khalal and Rutab stage were obtained from The Central Laboratory for Date palm Research and Development , Agriculture Research Center , Giza , Egypt.

-Chemicals

The chemicals were purchased from El-Gomhoria Company for medical materials, Cairo, Egypt.

Methods

- Technological Methods

Amhat date palm fruit (ripening operation)

Amhat dates (in khalal stage) were stored in freezing condition

At – 18 º C for one week , then transferred to oven at 40 º C for two h. the temperature was increased to 65º C for another two h. the moisture content would be in the range from 50.48 % (rutab stage).(**Nezam El-Din and Abd El- Hameed.,2003**) .

Analytical methods -

1-Determination of chemical composition

Moisture content , crude protein , crude fiber , ash content , fats , total acidity (as citric acid) and pH values were determined according to the methods of **A.O.A.C.(2016).**

2-Determine total soluble solids

The T.S.S were estimated by using an Atago digital refractometer at 25º C **(Ranganna., 2007).**

3-Identification and quantification of sugars by HPLC

The samples were fractionated and identified by HPLC "High Performance Liquid Chromatography " (column used was Phenomenex @ Luna NH2 250 × 4.6 mm, mobile phase was Acetonitrile : HPLC grade water 80:20 (v/v) , detector by RI detector and data integration by claritychrom@ software , (Food Safety and Quality control Lab., Fac.of Agric. Cairo Univ.) (HPLC smart line, Knauer, Germany) according to the method of **(Chinnici *et al*., 2005).**

4-Determine minerals content

Minerals content were determined using atomic absorption spectroscopy as reported by **A.O.A.C. (2016).**

5-Determine total free phenolic compounds

Total free phenolic compounds were determined colorimetrically by Folin – Ciocalteu reagent according to spectrophometric method described by **(Stratil *et al*., 2006) .**

6-Detemination of tannins

Tannins were estimated according to the method described by **Rebaya *et al* .,(2014).**

Tannins fractions (LMWT and HMWT) were determined by this method that reported by **Czochanska *et al* ., (1979).**

Condensed tannins were estimated by this method that reported by

(Iqbal *et al*., 2011)

7-Determine Pigments

Determination browning content

One gram of samples on dry matter were extracted by ethanol (60 %)

The color of clear extract measured to 420 nm as reported by **Ranganna , (2007).**

8-Determination of DPPH free radical scavenging activity (1,1-Diphenyl -2- Picrylhydrazyl) .

The antioxidant activity of sample extract was estimated in terms of hydrogen donating or radical-scavenging ability using the stable DPPH method as found by**'' Xu and Chang ., 2007 ''** The reaction mixture containing 1 ml of extract at concentration (150µg /ml) + 1 ml DPPH (0.2mM) was vigorously shaken and incubated in darkness at room temperature for 30 minutes .The absorbance was browse at 517 nm exploitation UV-visible photometer. Radical scavenging activity was expressed as percent of inhibition and was calculated exploitation the subsequent formula:

%DPPH= [Absorbance of Control – Absorbance of Sample / Absorbance of Control] × 100.

9-Identification and quantification of phenolic and flavonoid compounds by HPLC

Fractionation and identification of phenolic compounds were carried out by HPLC (Agilent 1260 infinity HPLC series(Agilent USA), equipped with Quaternary pump , akinetex® 5µm EVO C18 100 mm × 4.6 mm, (Phenomenex, USA), the

separation is achieved using a ternary linear elution gradient with (A) HPLC grade water 0.2% H_3PO_4 (v/v) , (B) methanol and (C) acetonitrile . The injected volume was 20 µl . Detection VWD detector set at 284 nm . (Food Safety and Quality control Lab., Fac.of Agric. Cairo Univ.) according to the method described by **Pascale *et al.* (1999),** while flavonoid compounds were carried out by HPLC (Aglilent 1260 infinity HPLC Series (Agilent, USA) equipped with Quaternary pump, a Zorbax Eclipe plus C18 column 150 mm × 4.6 mm id., (Aglient technologies , USA) Eluent : methanol : H_2O with 0.5% H_3PO_4 , 50:50with flow rate 1ml/min. the injected volume was 20µl. Detection ; UV detector set at 272 nm) (Food Safety and Quality control Lab., Fac.of Agric. Cairo Univ.) according to the method described by **Pirjo *et al.* (2000)**.

10-Biological Evaluation

Cytotoxic activity as anticancer

-Preparation of sample extracts

50 gram of dry matter of sample(date palm fruit) was extracted by suitable solvent (ethanol , methanol , and acetone) . The extract was filtered and evaporated by rotary evaporator at 45º C . The extract was stored at -18º C until using measurement of cytotoxicity for anticancer by SRB assay "Sulforhodamine B Colorimetric assay" It was used method method of" **Skehan *et al.*, 1990**" for determination of potential cytotoxicity.

Cells were coated in 96-multiwell plate (10^4 cells/well) for 24 hours before treatment with compounds or sample to allow attachment of cell to the wall of plate. Different concentration of the compounds or sample under test (0.0, 62.5 , 125 , 500 µg/ml) were added to cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds in atmosphere of 5% carbon dioxide for 48 hours at 37º C . Cells were fixed, washed and strained with sulfo-rhodamine-B stain after 48 hours. Excess stain was washed with 1% acetic acid and attached stain was recovered with Tris-EDTA buffer, an ELISA reader was used to measure color intensity by photometric determination of absorbance at 570 nm using microplate ELISA. Triplicate repeats were performed for each concentration and the average was calculated. Data were expressed as the percentage of relative viability compared with the untreated cells and the vehicle control, with cytotoxic indicated by < 100 relative viability.

Percentage of relative viability was calculated using the following equation:

(Absorbance of treated cells/ Absorbance of control cells)× 100

Then the half maximal inhibitory concentration (IC_{50}) was calculated from the equation of the dose response curve . **(Skehan *et al.*, 1990)** ."This experiment was conducted at the Egyptian Cancer Institute, Cairo, Egypt".

11-Measurement of antimicrobial activity

-Preparation of sample extracts

The samples "equal to 50 gram dry matter " was extracted by 100ml aceton "70 %" . Each extract was filtered and concentration by rotary evaporator at 25 º C . The extract kept at – 18º C until use **(Daoud *et al.*, 2015).**

The extract was tested against" two Gram positive bacteria" *Staphylococcus aureus* , *Bacillus subtilis* and" Gram negative bacteria " *Escherichia coli* , *Salmonella typhi* . The anti-fungal of the compounds were tested against fungus *"Aspergillus flavus* ".

Paper discs of Whatman filter paper were prepared with standard size (3mm) .And they were cut and sterilized in an autoclave . The extract solutions were added using micropipette 100 , 200 and 400 µl , the concentration of date palm fruit were 0.125 , 0.25 and 0.5 mg respectively . Previous volumes of extracts were added to the filter paper which was placed in middle of petri dishes contained nutrient agar media with individual microorganisms *Staphylococcus aureus* , *Bacillus subtilis* , *Escherichia coli* , *Salmonella typhi, Aspergillus flavus*)The petri dishes were brooded at 36◦C and the inhibition zones were recorded after 24 hrs. Each treatment was replicated three times. Ampicilin (1 mg/ml) was individually used as positive controls for bacteria and Clotrimazole was individually used as positive controls for fungi , antimicrobial activity was determined according to the method described by **Fadda and Hala (2013)** at Food Technology Research Institute , Agric. Res. Center , Giza , Egypt . The % activity index for the compounds was calculated by:

%Activity

$$= \frac{\textbf{\textit{Zone of inhibition by test compound (diametre)}}}{\textbf{\textit{Zone of inhibition of standard (diametre)}}} \times 100$$

12-Sensory evalution

Amhat date fruit samples were organoleptically tested for their colour , taste , odour , texture and general appearance using scale from 1 to 10 , panel test was done in Food Technology Research Institute**. (Barrett *et al.*, 2010) .**

13. Statistical Analysis

Data analysed with SPSS (Statistical Package for the Social Science) 20.0 for windows. The mean , SD of mean and LSD were calculated . The Data were analysed by one- way analysis of variance (ANOVA) . Duncan's multiple range test was used to separate means. Significance was accepted at a probability $P \leq 0.05$**.**

Results and Discussion

The physiochemical characteristics of amhat date palm fruit.

Data represent in **Table (1)** showed the physicochemical of amhat dates at khalal stage , natural and artificial rutab stage .It could noticed that the moisture content of khalal date was 72.06% which decreased by ripening to 55.39 % and 50.48% of natural rutab and artificial rutab of amhat date respectively. The pH value of khalal amhat date was 4.5 which increased to 5.5 and 5.7 of natural and artificial ripening respectively. Total acidity of khalal Amhat date was 0.56 % which changed to 0.45 % and 0.42 % of natural and artificial rutab dates .

Crude protein of amhat date were 1.53 , 1.57 and 1.61 % of khalal , natural and artificial ripening dates respectively that is due to loss moisture **Nasir *et al.*, (2014).**

Crud fiber of khalal amhat date was 14.46 % which decreased to 9.84 % and 9.59 % of khalal , natural and artificial ripening dates respectively. Lipid content of amhat dates were 0.38 , 0.36 and 0.36 % of khalal , natural and artificial ripening dates respectively **Al- Hooti *et al.* , (1997)** .

Ash content of khalal amhat date was 1.88 % which increased to 1.92 % and 2.05 % of natural and artificial ripening respectively.

Table 1. Physiochemical characteristics of khalal Amhat date , natural rutab and artificial rutab Amhat date .

Samples Items	Khalal	N. rutab	A. rutab	LSD
Moisture %	72.06 ± 1.80^{a}	55.39 ± 1.38^{b}	50.48 ± 1.26^{c}	$2.998^{0.05}$
Total solid T.S.%	27.94 ± 0.70^{c}	44.61 ± 1.12^{b}	49.52 ± 1.24^{a}	$2.084^{0.05}$
pH value	4.5 ± 0.11^{b}	5.5 ± 0.14^{a}	5.7 ± 0.14^{a}	$0.263^{0.05}$
Total Acidity %	0.56 ± 0.014^{a}	0.45 ± 0.011^{b}	0.42 ± 0.011^{c}	$0.024^{0.05}$
Crude protein %	1.53 ± 0.038^{b}	1.57 ± 0.039^{ab}	1.61 ± 0.04^{a}	$0.078^{0.05}$
Crude fiber %	14.46 ± 0.36^{a}	9.84 ± 0.25^{b}	9.59 ± 0.24^{b}	$0.575^{0.05}$
Total lipid %	0.38 ± 0.01^{a}	0.36 ± 0.009^{b}	0.36 ± 0.009^{b}	$0.018^{0.05}$
Total soluble solid %(T.S.S.)	21 ± 0.53^{c}	35 ± 0.88^{b}	41 ± 1.03^{a}	$1.668^{0.05}$
Ash %	1.88 ± 0.047^{b}	1.92 ± 0.048^{b}	2.05 ± 0.05^{a}	$0.098^{0.05}$

*All chemical composition measured on dry weight basis (DWB) except total soluble solids and total solids which measured on fresh weight basis (FWB). * The mean value with different superscript alphabets in rows indicate significantly differences ($p \leq 0.05$) using LSD TEST

2. **Fractionation of sugars content of Amhate date (khalal , Natural rutab and Artificial rutab using HPLC (High Performance Liquid Chromatography)**

Sugars content of amhate date variety by using HPLC for measuring the sugars content of Amhate date it was found that khalal , N. rutab and A. rutab contained 21.95 , 30.03 and 31.12 % ; 24.79 , 32.33 and 35.76 %; and 0.61, 2.13 and 1.67 % (DW) of fructose , glucose and sucrose respectively **(Table 2) .** So, The high content of fructose and glucose after ripening to rutab may be related to release of sugars from hydrolysable tannins and saponin by hydrolysis.as reported by **(Adeosun *et al.*, 2016)**

Table 2. Sugars fractionation by HPLC of Amhate date (Khalal , N. rutab and A. rutab)

Samples Items	Khalal	N. rutab	A. rutab
Fructose%	**21.95**	**30.03**	**31.12**
Glucose %	**24.79**	**32.33**	**35.76**
Sucrose %	**0.61**	**2.13**	**1.67**

*The percentage measured on dry weight basis .

3.Minerals content of khalal amhat date , natural and artificial rutab amhat date.

The minerals content of khalal amhat date , natural and artificial rutab amhat dates **(Table 3)** showed the micro and macro – element of amhat dates the iron(Fe of khalal amhat date was 2.11mg/100g which increased in nutral rutab amhat date and artificial amhat date were 2.22 and 2.39 mg/100g respectively .

Also Phosphorus (P) was increased by ripening, at khalal stage phosphorus was 110 mg/100g, in natural and artificial rutab were 120 and 132.61 mg/100g respectively.

Potassium , calcium and magnesium were decreased by ripening , Potassium content (K) of amhat date at khalal stage , natural and artificial rutab were 440 , 433 and 430 mg/100g respectively . calcium of khalal amhat date was 460 mg/100 , natural and artificial rutab amhat date were 380 and 371.85 mg/100g respectively . Magnesium of khalal amhat date was 270 mg/100g , natural and artificial rutab amhat date were 230 mg/100g and 231.90 mg/100g respectively. These results were agreement with **Al-Hooti *et al.*, (1997).**

Table 3. Minerals content (mg /100g) of khalal amhat date, natural and artificial rutab amhat date (on dry weight basis)

Minerals / Samples	Fe	P	K	Ca	Mg
Khalal Amhat date	2.11	110	440	460	270
Natural rutab	2.22	120	433	380	230
Artificial rutab	2.39	132.61	430	371.85	231.90

4. Phenolic compounds of amhat date.

From **Table (4)** it was cleared that total phenolic compounds of khalal amhat dates were 2.86 % which decreased by natural ripening (rutab) to 1.524 % and by artificial ripening to 1.817 % .The decrease of total phenolic compounds may be related to enzymatic browning by polyphenol oxidase as found by **Parr and Bolwell (2000) .** So high significant difference between total phenolic compound of khalal amhate date and the others rutab dates.

Simple phenolic compounds of khalal dates were 1.330 % but Simple phenolic compounds after natural ripening of amhat date was 1.318 % and after artificial ripening of amhat date was 0.640 % the clear decrease after artificial ripening may be related to the effect low heating on acceleration of enzymatic and non- enzymatic browning . A significant difference of simple phenols was found between khalal amhate date and artificial repining.

Hydrolysable tannin of khalal date was 1.370 % but for natural rutab was 0.950 % and artificial rutab was 1.160 % . The increase of artificial rutab than natural rutab resulted from the effect of heat during ripening by oven on inhibition of enzymatic hydrolysis of hydrolysable tannins and degradation of condensed tannin . Low molecular weight of condensed tannin of khalal date was 0.4936 % which decreased to 0.4714 and 0.4072 % of natural rutab and artificial rutab respectively . High molecular weight of condensed amhat tannin was 1.6544 % , This concentration decreased by ripening to 0.9100 and 0.6095 % of natural rutab and artificial rutab respectively .The high decrease of artificial ripening resulting from the effect of heating on degradation of condensed tannins but natural ripening was affected by enzymatic hydrolysis only. From **(Table 4)** it was cleared that soluble and condensed tannins showed high significant difference than N. rutab and A. rutab.

So, The decreases of total phenolic compounds may be related to role of the browning reaction (enzymatic for natural amhat date and non – enzymatic for artificial amhat date). Also a clear decreases in hydrolysable tannin of natural amhat than artificial amhat by enzymatic hydrolysis as reported by **(Tanaka *et al*., 1994)** but condensed tannin (LMW and HMW) of natural rutab exhibited more decrease as showed in **Table (4)** these results coming from the effect of heating.

Table 4. Phenolic compounds of (mg /100g) of khalal Amhat date, natural and artificial rutab Amhat date (on dry weight basis)

Samples / Items	**Khalal Amhat date**	**Natural rutab Amhat date**	**Artificial rutab Amhat date**	**LSD**
Total phenolic compound	2.860 ± 0.072^{a}	1.524 ± 0.038^{c}	1.847 ± 0.046^{b}	$0.1076^{0.05}$
Simple phenols	1.330 ± 0.033^{a}	1.318 ± 0.033^{a}	0.640 ± 0.016^{b}	$0.0571^{0.05}$
Hydrolysable tannin	1.370 ± 0.034^{a}	0.950 ± 0.024^{c}	1.160 ± 0.029^{b}	$0.0586^{0.05}$
Condensed tannin	2.1481 ± 0.054^{a}	1.39416 ± 0.035^{b}	1.0767 ± 0.027^{c}	$0.080^{0.05}$
LMW	0.4936 ± 0.0026^{a}	0.4714 ± 0.0019^{b}	0.4072 ± 0.0018^{c}	$0.0039^{0.05}$
HMW	1.6544 ± 0.0252^{a}	0.9100 ± 0.0091^{b}	0.6095 ± 0.0078^{c}	$0.031^{0.05}$

The mean value with different superscript alphabets in rows indicate significantly differences ($p \leq 0.05$) using LSD TEST.

5. The browning content on Amhat date.

From **Table (5)** it is clear that the browning reaction is occurred in natural Amhat date more than artificial Amhat date because enzymatic effect by polyphenol oxidase had good role for change the color through browning reaction (enzymatic browning) but artificial Amhat date showed a less browning color non – enzymatic browning reaction between amino acids
(tyrosine , phenylalanine) and sugars **(Nezam El – Din and Abd El – Hameed., 1997) .**

Table 5. Browning content measured as optical density at 420 nm of amhat date .

Samples	Optical density 420 nm
Amhat date (khalal stage)	**0.257**
Amhat date (natural rutab)	**0.792**
Amhat date (artificial rutab)	**0.714**

*The color measured as optical density per 1 gm dry matter.

6. The antioxidant capacity of Amhat date

From **Table (6)** it was found that total antioxidant capacity of khalal amhat date was 1297.119 mg / 100 g , 1293.877 mg / 100g and 372.9 mg / 100g for natural and artificial ripening of amhat dates .

So , the total antioxidant capacity very high in khalal date which composed of phenolic compounds , hydrolysable tannins (gallo and ellagi tannins) and condensed tannins (low and high molecular weight tannins) .

So, A significant difference between khalal date and artificial ripening very high, also khalal date showed a high significant difference more than artificial ripening.

A high significant difference between natural ripening and artificial ripening. No significant difference between khalal date and natural ripening .

Poly phenolic compounds effect on antioxidant capacity related to their hydroxyl content of their aromatic ring which lead to chelate and scavenging the free radicals**.(Dekok *et al*., 2008)**

Table 6. Total antioxidant capacity of amhat dates (mg/100g) as ascorbic acid equivalent on fresh weigh basis .

Samples Items	Khalal	Natural ripening rutab	Artificial ripening rutab	LSD
Total antioxidant capacity	1297.119 ± 64.856^{a} (mg/100g) as ascorbic acid equivalent	1293.877 ± 64.694^{a} (mg/100g) as ascorbic acid equivalent	372.9 ± 18.645^{b} (mg/100g) as ascorbic acid equivalent	$107.83^{0.05}$

* The mean value with different superscript alphabets in rows indicate significantly differences ($p \leq 0.05$) using LSD TEST

7. Fractionation of phenolic compounds content by HPLC (High Performance Liquid Chromatography).

From **Table (7)** it was appeared that the maximum Phenolinc compounds of khalal dates were pyrogallol (122.344 mg / 100 g) , Gallic acid (80.394 mg / 100g), Catechol (10.276 mg / 100g) , Chlorgenic acid (30.147 mg / 100 g), Caffeic acid (25.289 mg / 100 g) Vanillin (2.033 mg / 100 g) and Ellagic acid
(377.377 mg / 100 g). And the maximum phenolic compound of natural ripening (rutab) were P-hydroxy benzoic acid (24.421 mg / 100g), Syringic acid (5.745 mg / 100g) , Caffeine (51.865 mg /100 g) P- Coumaric acid (2.074 mg / 100 g) Ferulic acid (5.259 mg / 100 g) and Salicylic acid (13. 273 mg / 100 g) and Cinnamic acid (5.180 mg / 100 g) . The maximum phenolic compound of artificial ripening of Amhat dates were Quinol (68.798 mg / 100 g) , Vanillic acid (17.787 mg / 100 g) and Benzoic acid (33.479 mg / 100 g). The important role of phenlic compounds is inhibition of cellular proliferation. **(Kuntz *et al*., 1999)**

Table 7. Fractionation of phenolic compounds content (mg / 100 g on dry weight basis) by HPLC (High Performance Liquid Chromatography) of amhat date.

Samples Items	Khalal	Natural rutab	Artificial rutab
Pyrogallol	122.344	54.461	ND
Quinol	ND	ND	68.798
Gallic acid	80.394	10.504	0.927
Catechol	10.276	7.460	1.551
P- hydroxy benzoic acid	12.770	24.421	1.769
Caffeine	ND	51.865	2.289
Chlorogenic acid	30.147	4.750	0.466
Vanillic acid	ND	1.769	17.787
Caffeic acid	25.289	2.154	0.660
Syringic acid	3.712	5.745	0.454
Vanillin	2.033	1.885	0.561
P-coumaric acid	0.565	2.074	0.876
Ferulic acid	ND	5.259	0.584
Benzoic acid	ND	ND	33.479
Ellagic acid	377.377	ND	14.198
O- coumaric acid	ND	ND	0.456
Salicylic acid	6.077	13.273	0.204
Cinnamic acid	3.829	5.180	0.743

*ND: Not Detected

8. Fractionation of flavonoid compounds content by HPLC (High Performance Liquid Chromatography)

The maximum flavonoids of khalal dates were Myricetin (488.636 mg / 100g) and Rutin (42.409mg / 100g) and for natural rutab dates were Neringein (86.705 mg / 100g) , Rosemarinic (75.05 mg / 100g) , Myricetin (47.480 mg /100 g) and Kampherol (27.705 mg / 100g) but artificial ripening rutab contained only Myricetin (20.357 mg / 100g) and Rutin (7.888 mg / 100 g) which were less than the others . **(Table 8)**

Table 8. Fractionation of flavonoid compounds content (mg / 100 g) on dry weight basis by HPLC (High Performance Liquid Chromatography) .

Samples Items	Khalal	Natural rutab	Artificial rutab
Myricetin	488.636	47.480	20.357
Quercitin	ND	7.187	ND
Rosemarinic	ND	75.050	ND
Neringein	ND	86.911	ND
Kampherol	ND	27.705	ND
Rutin	42.409	36.413	7.888

*ND: Not Detected

(Quercitin , rutin and myricetin) and cumaric , gallic , ellagic , ferulic and hydrolysable tannins) prevent cancer and inflammatory disease . A positive correlation between polyphenolic content and cancer reduction**. (Carocho and Ferreira.2013).**

9. Biological Evaluation

Cytotoxic effect as anticancer

By measuring the effect of Amhat date extracts on human liver carcinoma cell line (HEPG 2) for studying in vitro its effect as cytotoxic **(Table 9 and 10)** (**Figure 1 and 2)**, it was found that using some solvents (methanol 100 % , methanol 75 % and acetone 70 %) to extract khalal , natural rutab and artificial rutab exhibited lower IC_{50} (concentration lead to decrease cell viability to 50 %) Than ethanol .

These results revealed that methanol 100 % extracted saponin , methanol 75 % extracted total phenolic compounds (**Yuliana *et al*.,2014**) and acetone 70 % extracted total tannin (**Makkar , 2003).**

So, the best IC_{50} was natural rutab which led to the lowest IC_{50} (153 µg / ml) for inhibition of the liver carcinoma cell line (HEPG 2).

Table 9. Cytotoxic effect of Amhat dates fruit against human liver cancer cell lines (HEPG2) extraction by ethanol 70 %.

Solvent	Samples	Concentration µg/ml					IC_{50}
		0	62.5	125	250	500	
Ethanol alcohol 70 %	**Khalal**	100.0	77.0	65.0	50.0	45.1	250
	N. Rutab	100.0	62.2	60.9	59.5	45.4	157
	A.Rutab	100.0	88.4	70.4	40.3	45.9	205

IC_{50} : Concentration able to decrease cell viability by 50 % versus control culture.

Table 10. Cytotoxic effect of Amhat dates fruit against human liver cancer cell lines (HEPG2) extraction by methanol 100%, methanol 75 % and acetone 70%.

Solvent	Samples	Concentration µg/ml					IC_{50}
		0	62.5	125	250	500	
Methanol 100 %, methanol 75% and acetone 70 %	**Khalal**	100.0	71.1	67.0	55.4	58.6	230
	N. Rutab	100.0	66.4	55.4	41.1	39.0	153
	A.Rutab	100.0	93.3	74.5	39.9	44.6	207

IC_{50} : Concentration able to decrease cell viability by 50 % versus control culture.

Also, **Table (7)** illustrated that Syringic acid , P-Qumaric acid , Ferulic acid ,Salicylic acid and Cinnamic acid represented the maximum concentration for natural rutab which may by one and for more are the responsible phenolic compounds for inhibition effect for liver carcinoma cell line . And from flavonoids one and more (Quercitin , Rosemarinic , Neringein , Myricetin and

Kampherol) are responsible for inhibition of cancer cells**. (Bawadi *et al* , 2005 ; Ramos, 2008 ; Wang *et al* , 2013 ;and Subramanin *et al* , 2016)**

The lowest IC_{50} (extracted by ethanol and some solvents) was found for natural rutab which represent (157 µg/ml) and (153µg/ml) respectively , this meaning that natural ruab is better than the others to inhibition the liver cancer cell (HEPG 2) .

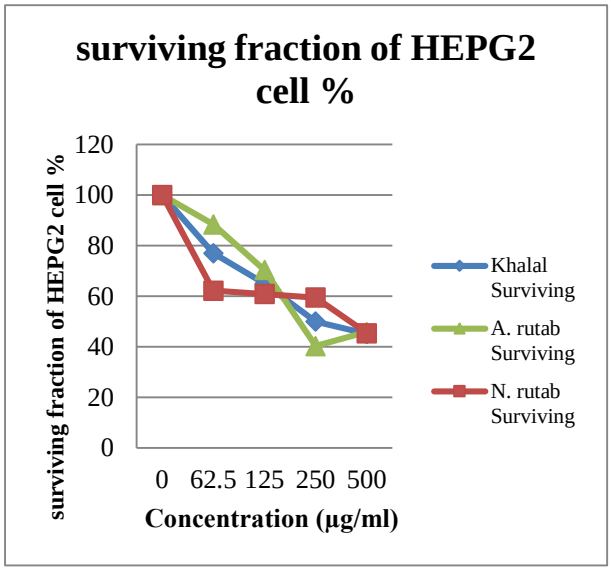
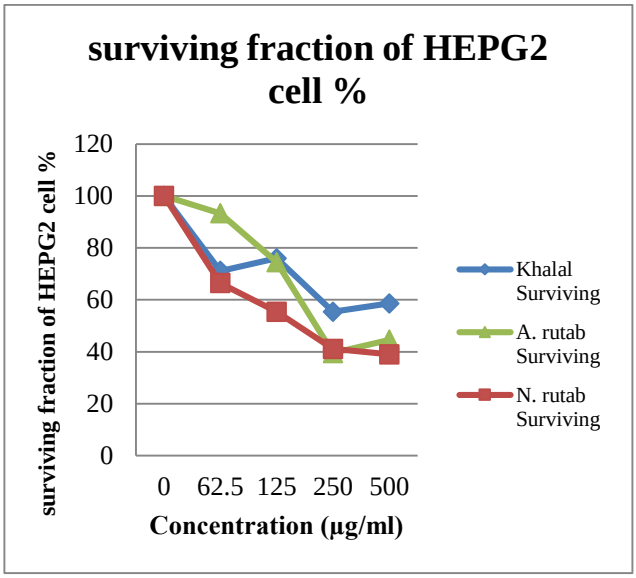


Figure (1): Cytotoxic effect of Amhat dates fruit against human liver cancer cell lines (HEPG2) extraction by ethanol 70 % .



figure(2): Cytotoxic effect of Amhat date against human liver cancer cell extraction bymethanol 100%, methanol 75% and acetone 70%

The responsible change of every compounds of anticancer: By comparing the phenolic compounds (**Table 7**) to the IC_{50} of Amhat date (Khalal , Natural and artificial ripening) it appeared that one or more of these phenolic compounds (caffeine , p-coumaric acid and ferulic acid) are responsible for enhancing IC_{50} and act as anticancer agent **.(Alias *et al*., 2009).**

Coumaric group have antitumor activity to certain extent this group such as coumarin , 4-hydroxy coumarin , 7-hydroxycoumarin, dicoumarin , psoralidin ,warfarin , dephetin and coumarin aesculetin . These compounds have been studied for anticancer properties . coumarin and its derivatives have activity against human pancreatic . **(Devji *et al*.,2011**). SO, it is may be have a synergetic effects of some phenolic compounds on IC_{50} of Amhat date to have clear effects more than one phenolic compound alone.

10. Microbiological Evaluation Effect of Amhat date extracts as antimicrobial agents.

From **Table (11)**, it was found that the extract of Khalal Amhat date did not exhibited any effect on all micro – organisms by using concentration 100 µl but the concentration 200 µl had positive effects on *E. coli* and *Salmonella typhi* with growth inhibition reached to 2.7 cm and 3.0 cm respectively .

Also , the concentration 400 µl led to more inhibition of micro – organisms growth which reached to 3.0 , 3.7 and 2.0 cm for *E. coli* , *Salmonella typhi* and *Staphylococcus aureus* respectively but no effect appeared for the others .

Natural Rutab date extract with concentration 100 µl had positive effect only with *Salmonella typhi* and its inhibition growth of diameter reached to 2.8 cm but the double concentration (200 µl) led to positive effect on *E. coli* , *Salmonella typhi* and *Staphylococcus aureus* and inhibition effects reached to 3 , 3.5 and 2.1 cm respectively , also the maximum concentration (400 µl) exhibited high inhibition effects on *E. coli* , *Salmonella typhi* and *Staphylococcus aureus* which reached to 4,4 and 2.3 cm respectively .(**Saleh *et al*.,2013**)

Artificial ripening of amhat date (A. Rutab) extract with concentration 100 µl had positive effect on *E. coli* and *Salmonella typhi* and the inhibition of previous micro – organisms reached to 3.3 and 3.2 cm respectively . But the other micro – organisms did not exhibited any inhibition as showen .

In **Table (11)** By measuring the concentration of artificial rutab extract to be 200 and 400 µl led to increase the effect of inhibition of *E. coli* from 3.4 to 4.0 cm respectively and inhibition *Salmonella typhi* from 3.3 to 4.0 cm respectively .

It is clear from **Table (11)** that the significant difference increase by increasing the concentration from 100 µl to 200 µl to 400 µl as shown for *E. coli* , *Salmonella typhi* and Staph.**(Qadoos *et al*.,2017).**

The strongest effect of A. rutab extract on E. coli may be related to A. rutab extract contain more amounts of vanillic acid than these found in N. rutab date. **(Naz *et al*.,2006).**

Table 11. The antimicrobial effect of amhat date extracts (Zone diameter of inhibition,cm)

Samples	Conc.	*E. coli* ATCC25922	Salmonella ATCC19930	*Staph. aureus*. MRSA ATCC43300	*B. subtilis* ATCC6051	*Asperigillus flavus* CAICC41
Khalal	**100µl**	NO	NO	NO	NO	NO
	200µl	2.7 ± 0.068^{b}	3 ± 0.075^{b}	NO	NO	NO
	400µl	3 ± 0.075^{a}	3.7 ± 0.093^{a}	2 ± 0.050^{a}	NO	NO
LSD		**$0.116^{0.05}$**	**$0.138^{0.05}$**	**$0.058^{0.05}$**	———	———
N. Rutab	**100µl**	NO	2.8 ± 0.070^{c}	NO	NO	NO
	200µl	3 ± 0.075^{b}	3.5 ± 0.088^{b}	2.1 ± 0.053^{b}	NO	NO
	400µl	4 ± 0.10^{a}	4 ± 0.10^{a}	2.3 ± 0.058^{a}	NO	NO
LSD		**$0.144^{0.05}$**	**$0.173^{0.05}$**	**$0.090^{0.05}$**	———	———
A. Rutab	**100µl**	3.3 ± 0.083^{c}	3.2 ± 0.080^{c}	NO	NO	NO
	200µl	3.4 ± 0.085^{b}	3.3 ± 0.083^{b}	NO	NO	NO
	400µl	4 ± 0.10^{a}	4 ± 0.10^{a}	NO	NO	NO
LSD		**$0.179^{0.05}$**	**$0.176^{0.05}$**	———	———	———

*****NO : Not Observed * each 100 µl contain of 0.125 mg of samples extract * The mean value with different superscript alphabets in rows indicate significantly differences ($p \leq 0.05$) using LSD TEST

12. Sensory Evaluation

The organoleptic evaluation (**Table 12**) illustrated that no significant difference between natural and artificial ripening as shown in color, this result may be related to small difference in browning content of natural and artificial rutab (**Table 5**) also, no significant difference between natural and artificial ripening as shown in general appearance but a significant difference in taste, odor and texture . Significant difference in texture may be related to the inhibition of pectinase enzyme (enzyme responsible for smooth) as effect of heat used to obtain artificial rutab . (**Pedrolli et al., 2009**). In general, artificial rutab amhat dates are sensually acceptable .

Table 12. Sensory evaluation of Natural and Artificial ripening amhat date

Test	Samples	Values	LSD
Color	Natural ripening	9.40 ± 0.737^{a}	$0.824^{0.05}$
	Artificial ripening	8.80 ± 1.373^{a}	
Taste	Natural ripening	10.00 ± 0.00^{a}	$0.653^{0.05}$
	Artificial ripening	8.33 ± 1.234^{b}	
Odor	Natural ripening	10.00 ± 0.00^{a}	$0.326^{0.05}$
	Artificial ripening	9.67 ± 0.617^{b}	
Texture	Natural ripening	10.00 ± 0.00^{a}	$0.339^{0.05}$
	Artificial ripening	8.87 ± 0.640^{b}	
General appearance	Natural ripening	9.60 ± 0.507^{a}	$0.500^{0.05}$
	Artificial ripening	9.27 ± 0.799^{a}	

* The mean value with different superscript alphabets in rows indicate significantly differences ($p \leq 0.05$) using LSD TEST

Conclusion:

Amhat date palm is rich by sugers , minerals , fiber and bioactive component (phenolic compounds and tannins) in high levels. Date palm plays a good role as antioxidant, anticancer and antimicrobial agent, therefore recommended consumption in all stages.

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التقييم الغذائي و النشاط المضاد للأكسدة و المضاد للسرطان و الميكروبات لصنف البلح الامهات المصري

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بلح الامهات (من الاصناف الرطبة) من اكثر الفاكهة شعبية بمصر و تنتج مصر اصناف رطبة و نصف جافة و جافة و معظم الانتاج من الاصناف الرطبة و لذلك كان من الضروري ترطيب البلح الامهات صناعيا للإطالة فترة صلاحيته و منع ظهور طعم غير مقبول به و عليه تم تقدير التركيب الكيميائي للبلح الامهات (الرطوبة- المواد الصلبة الذائبة – البروتين – الدهون –الالياف الخام) و ايضا تم تقدير بعض العناصر المعدنية و القيمة الغذائية للبلح الامهات في مرحلة الخلال و مرحلة الرطب و كذلك ثمار التي تم انضاجها صناعيا تم تقدير المواد النشطة بيولوجيا (المركبات الفينولية الكلية – الفينولات البسيطة – التانينات الكلية – التانينات الذائبة- التانينات المعقدة) و تم تفريد كلا من السكريات و المركبات الفينولية و الفلافونيدات باستخدام جهاز الفصل الكروماتوجرافي السائل العالي الاداء لما لهذه المركبات من دور هام كعامل مضاد للاكسدة و السرطان ومثبطة للميكروبات وتم اختبار دور هذه المركبات كمضاد للسرطان باختبار IC_{50} و كذلك اجراء اختبار التثبيط الميكروبي (قياس قطر التثبيط لمستخلص البلح الامهات الخلال و الرطب و الذي تم ترطيبه صناعيا) ووجد بعض الاحماض الفينولية الهامة مثل الكيوماريك و الفريوليك و الكافيين و التي قد يكون لها تأثير – منفردة او مجتمعة – كمضاد للسرطان و مثبط لنمو الاحياء الدقيقة.