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Studies on Biological Effect of some Selected Foods (Un-Pollinated Siwi Date, Date Palm Pollen and Doum Fruit)

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ABSTRACT

The current work aimed to study the biological effects of fresh un-pollinated date, date palm pollen and doum fruit against some cancer cells line and some pathogenic microorganisms. Chemical composition including moisture content, total soluble solids, ash content and total and reducing sugars were determined. Also, antioxidant compounds such as phenolic compounds, hydrolysable tannins, condensed tannins and flavonoids were measured. The biological effects of fresh un-pollinated date, date palm pollen and doum fruit extracts against some human cancer cells include colon (HCT), prostate (PC3), breast (MCF7) and larynx (HEP2) cancers were tested. In addition, the antimicrobial effect of these extracts against some pathogenic microorganisms includes bacteria, mold and yeast were examined. The results indicated that fresh un-pollinated Siwi date had the highest content of antioxidant compounds compared with date palm pollen and doum fruit, therefore lead to the high antiradical activity. On the other hand, the date palm pollen had the highest content of some minerals content such as calcium, magnesium, iron, zinc and manganese compared with fresh un-pollinated date and doum fruits. The best effect against the four human cancer cells has been observed with fresh un-pollinated date extract. The cancer cells viability has been decreased up to 50% when the fresh un-pollinated date extract was used with concentration 62 µg/ml approximately. However, the highest decreased of cancer cells reached to 25% approximately when a concentration of the extracts has been used between 125 and 250 µg/ml. Also, fresh un-pollinated date revealed the best effect against gram negative bacteria but date palm pollen exhibited the best effect against gram positive bacteria, mold and yeast. So, the obtained data of the present study pointed that we neglected a very important bio-active products.

Keywords: (cancer cells, Antimicrobial agents, antioxidant compounds)

INTRODUCTION

Egypt is considered the main producers of dates in the Middle East which represent 17% of the world production (Saleh *et al.,* 2011). The worldwide production of date (*Phoenix dactylifera)* fruit is about 7.5 million tones and the first country for date production is Egypt which followed by Iran then Algeria and Saudi Arabia produced at 2014 (FAO, 2016). Date palm (*Phoenix dactylifera* L.) is belonging to the family *Arecaceae*. It is one of the oldest cultivated plants and widely distributed in various arid and semi-arid regions of the world including North Africa, Arabian countries and the Middle East. Date palm fruit extracts showed a wide range of effective properties such as antifungal, hepatoprotective activities, anti-inflammatory and anti-apoptotic activities. The date fruit components have shown the antitumor activity (Metwaly *et al.,* 2014). Unknown date is considered the main source of un-pollinated date compared with other un-pollinated dates of known varieties because the basic of unknown dates is made up of un-pollinated date. It is represent 5% from total production. Un-pollinated Khalal dates are rejected of a high bitter taste so that un-pollinated date (shees) must be leaved on palm tree until the end of khalal stage to get on the best qualities (Nezam El Din, 1996). Extracts of date fruit had been inhibited $H_2O_2^-$induced cell damage and apoptosis in a concentration dependent manner in cell lines against free radical induced cell death (Asadi-Shekaari *et al.,* 2008). Date fruit contained many bioactive components such as tannins, sterols, carotenoids and polyphenols especially phenolic acids, isoflavons, lignans and flavonoids (Al-Farsi and Lee, 2008). Date palm pollen is produced by male flowering date palm plants. Fresh date palm pollen is containing different concentrations of nutrients and phytochemical compounds such as polyphenols, flavonoids, minerals, vitamins, sugars, lipids, growth factors, certain antibacterial activities and over one hundred kinds of enzymes (Hassan, 2011). Date palm pollen played an important role in traditional herbal medicine through its role as antimicrobial, anti-inflammatory, antioxidant, antitoxic and hepato-protective activities (Le Blanca *et al.,* 2009).

Doum fruit is a palm native to Egypt, India and sub-Saharan Africa. It is an important source of dietary fibers, carbohydrates, antihypertension substances, nutritional trace minerals, protein and antioxidant compounds (Kamis *et al.,* 2003). Doum fruit have possessed good functional properties which can be used for various important applications in food industry (Aboshora *et al.*, 2014).

Cancer is an economic burden worldwide and a multi-factorial disease. Numerous chemo preventive agents were cured various types of diseases including cancer. These drugs are showed adverse side effect through alteration in gene normal action. The current treatment depended on radiotherapy and chemotherapy which are effective but also show adverse consequences. Components of plants such as polyphenols and flavonoids play a high important role in cancer control by the regulation of genetic pathways without any side effect (Gali-Muhtasib *et al.,* 2006). Plant extract have some different phytochemicals that interact in synergistic manner to exert their benefits to fight against multi-stage carcinogenesis process. Also, it is important to sperate some components of the plant extract and studying their effects on both normal and cancer cell lines but the separation steps lead to lose the activities of these compounds and their useful properties. The whole extract has the highest inhibitory effects on various cancer cells (Shahraki *et al.,* 2015).

The incidence of drug resistance against microbial pathogens is increasing significantly worldwide. Bacterial resistance against antimicrobial agents is one of the major difficulties in treatment. The present mode of treatment of bacterial infection is depending on antibiotics, which is expensive and also causes adverse side effects. Natural products and their constituents are a good in the control of infection as they are inexpensive, effective without side effects. A high effect in the prevention of bacterial diseases was played by *Phoenix dactylifera* and its constituents (Bokhari and Perveen, 2012). This study aimed to maximize the usefulness of un-pollinated date (shees), date palm pollen and doum fruit as nutritional, biological roles (anticancer cells as cytotoxicity) and antimicrobial agents as well as

identify their active components which are responsible of cancer inhibitions and antimicrobial activities. Trying to decide their responsible component of cancer inhibition and antimicrobial effects.

MATERIALS AND METHODS

Materials

Fresh un-pollinated date was obtained from Alhwamdya farm, Giza governorate, while doum fruit and date palm pollen were obtained from Aswan governorate, Egypt.

Methods

Analytical Methods

Gross chemical composition

Moisture, ash and acidity contents were determined according to A.O.A.C (2010). Total and reducing sugars were determined using the method of Somogy (1952). Minerals contents were determined using atomic absorption spectroscopy (Perkin Elmer 372, Japan) as described in A.O.A.C (2010) and pH values were measured by using Beckman pH meter with glass electrode at 25°C.

Determination of total soluble solid (TSS)

Total soluble solids were determined using an Atago DR-A1 digital refractometer (Atago Co. Ld., Japan).

Determination of total phenolic compounds

Total phenolic compounds was determined (as gallic acid aquivalent) of all samples extracts according to the method of Daniel and George (1979).

Determination of Hydrolysable tannins

Tannins were determined according to the method of Rooney *et al.,* (1981). Sample (2 g) was shaken on an Eberbach shaker with 50 ml of 1 % HCl in methanol for 24 hours at ambient temperature. Then, 1 ml was reacted with 5 ml of vanillin reagent (50:50 mixtures of 4% vanillin/ 8% HCl in methanol) for 20 min and absorbance was read at 500 nm. Tannic acid standard curve from 0.0 -1.0 mg/ml in 0.2 mg increments was used in calculating tannin levels .The amount of tannins is expressed as g tannic acid/100g DW.

Determination of condensed tannins

Condensed tannins were determined according to the procedure of Bate-Smith, (1973) at Food Technology Research Institute, Agric. Res. Center, Giza, Egypt. Extract (0.5 ml) is heated with 3.0 ml 5 % n-butanol-HCl for 70 min at 90-95 °C. The absorbance of the anthocyanidin formed is measured at 550 nm in the case of cyanidin, in the absence of precise information with regard to the degree of polymerization of the LA, the value $E_{1cm}^{1\%}$ is assumed to be 150.

Determination of radical scavenging activity (DPPH %)

The free radical capacity was carried out as reported by Mansouri *et al*., (2005), at Food Technology Research Institute, Agric. Res. Center, Giza, Egypt. The sample (10g) was extracted by 100 ml methanol 80% then 60 µl was taken from the extract to add to 1500 µl of DPPH solution (6x 10^{-5}M). After 30 min in the dark at a room temperature, the discoloration of the mixture was measured against a blank at 515 nm. The DPPH radical scavenging was calculated as

DPPH scavenging activity%= $A_B - A_S / A_B$ x100, where A_B = Blank= all reagents except sample and A_S = the absorbance of extracts.

Fractionation of phenolic compounds of extracts

Phenolic compounds of extracts were fractionated by HPLC (High Performance Liquid Chromatography) (Hewlett Packaged software) according to the method described by Goupy *et al.* (1999) at Food Technology Research Institute, Agric. Res. Center, Giza, Egypt.

Fractionation of flavonoid compounds of extracts

Flavonoid compounds were fractionated by HPLC (High Performance Liquid Chromatography) (Hewlett Packaged software) according to the method described by Mattila *et al.* (2000) at Food Technology Research Institute, Agric. Res. Center, Giza, Egypt.

Biological Evaluation

Anticancer activity as cytotoxicity

Prepration of extracts

Extracts were prepared from fresh of un-pollinated Siwi date, date palm pollen and doum palm fruit. The samples (50g) as dry matter were extracted by 500 ml ethanol (70%). Each extract was filtrated and then evaporated by using a rotary vacuum evaporator at 45°C. The extracts were kept in light protected containers at -18°C until use with some modification (Rasha and Faten, 2015).

1- Determination of potential cytotoxicity for anticancer drug screening

Potential cytotoxicity of the compounds was determined using the method of Skehan *et al.,* (1990). Cells were plated in 96-multiwell plate (10^4cells/well) for 24 hours before treatment with the compounds to allow attachment of cell to the wall of the plate. Different concentration of the compounds under test (0, 62.5, 125, 250, 500 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hours at 37°C and in atmosphere of 5% CO_2. 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, Color intensity was measured in an ELISA reader and the relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell lines (Colon, Prostate, Breast and Larynx carcinoma cells line) after the specified compound. This experiment was conducted at Egyptian Cancer Institute, Cairo, Egypt.

Determination of antimicrobial activity

Prepration of extracts

Extracts were prepared from fresh of un-pollinated Siwi date, date palm pollen and doum palm fruit. The samples (50g) as dry matter were extracted by 100 ml ethanol (70%). Each extract was filtrated and then evaporated by using a rotary vacuum evaporator at 45°C. The extracts were kept at -18°C until use (Daoud *et al.,* 2015).

2- Measurement of antimicrobial activity

The anti-bacterial activity of the extract was tested against a panel of two gram positive bacteria (*Staphylococcus aureus , Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli , Pseudomonas aeuroginosa)*. The anti-fungal activities of the compounds were tested against two fungi (*Candida albicans , Aspergillus flavus*). Extract was dissolved in DMSO (DiMethyl Sulfoxide) to prepare solutions with 12.5, 25, 50, 100 mg/ml concentration. Paper discs of Whatman filter paper were prepared with standard

size (3mm) which cut and sterilized in an autoclave. The extract solution (1 ml) with the desired concentrations of the complex solution were placed aseptically in the petri dishes containing nutrient agar media seeded with individual microorganisms (*Staphylococcus aureus , Bacillus subtilis , Escherichia coli, Pseudomonas aeuroginosa , Candida albicans* and *Aspergillus flavus*). The petri dishes were incubated at 36°C and the inhibition zones were recorded after 24 h of incubation. Each treatment was replicated three times. Ampicillin and Clotrimazole were used as standard antibacterial and antifungal agents, respectively. Ampicillin and Clotrimazole were prepared in DMSO at concentration of 1mg/mL. 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 the formula:

$$\%\ Activity\ Index = \frac{Zone\ of\ inhibition\ by\ test\ compound\ (diametre)}{Zone\ of\ inhibition\ by\ standard\ (diametre)} X100$$

Statistical analysis

Data of the present study were analyzed statistically and the differences between the means of treatments were considered significantly when they were more than least significant differnces (LSD) at the confidence level of 5% using the analysis of variance (ANOVA) by using Costat windows program (Costat program ver. 6.303, 2004).

RESULTS AND DISCUSSION

Chemical composition of fresh un-pollinated Siwi date, date palm pollen and doum fruit

The results in Table (1), show that the highest moisture content was determined in fresh un-pollinated date at the end of Khalal stage (59.56%) followed by date palm pollen (8.14%) then doum fruit (4.41%). Total soluble solids (T.S.S) of fresh un-pollinated date, date palm pollen and doum fruit were 28.43, 6.66 and 47.70% on fresh weight basis, respectively. So, date palm pollen was recorded the lowest content of total soluble solids. Also, total acidity was the highest in date palm pollen (0.30%) followed by fresh un-pollinated date (0.26%) then doum fruit (0.14%) but pH value was the highest in doum fruit (5.76) followed by fresh un-pollinated date (5.18) then date palm pollen (4.75) (Table 1). Ash content was the highest in date palm pollen which recorded (4.99%) and this data was in agreement with Hassan (2011) but it had the lowest content of total and reducing sugars (2.65 and 3.78%, respectively).

Table 1. Chemical composition of fresh un-pollinated Siwi date, date palm pollen and doum fruit

samples / composition	Fresh un- pollinated date	Date palm pollen	Doum fruit	$LSD_{0.05}$
Moisture (%)	59.56 ± 0.36 [a]	8.14 ± 0.71 [b]	4.41 ± 0.28 [c]	0.974
Total Solids (T.S) (%) (on FWB)	40.45 ± 0.36 [c]	91.86 ± 0.71 [b]	95.59 ± 0.28 [a]	0.974
Total Soluble Solids (T.S.S) (%) (on FWB)	28.43 ± 0.30 [b]	6.66 ± 0.13 [c]	47.70 ± 0.20 [a]	0.446
Total sugars (%)	56.09± 0.2 [a]	3.78 ± 0.1 [c]	26.85 ± 0.2 [b]	0.359
Reducing sugars (%)	49.121 ± 0.2 [a]	2.65 ± 0.2 [c]	20.17 ± 0.2 [b]	0.406
Ash (%)	2.14 ± 0.07 [c]	4.99 ± 0.07 [a]	3.18 ± 0.09 [b]	0.160
Total acidity (%) (as malic acid)	0.26 ± 0.01 [b]	0.30 ± 0.01 [a]	0.14 ± 0.02 [c]	0.0103
pH value	5.18 ± 0.09 [b]	4.75 ± 0.06 [c]	5.76 ± 0.09 [a]	0.0053

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

*** All Chemical properties measured on dry weight basis (DWB) except total soluble solids and total solids which measured on fresh weight basis (FWB)**

Minerals content of fresh un- pollinated Siwi date, date palm pollen and doum fruit

Data in Table (2) showed that the minerals content of fresh un- pollinated Siwi date, date palm pollen and doum fruit and it was observed that date palm pollen had the highest content of calcium, magnesium, zinc, iron and manganese (491.82, 185.61, 6.09, 9.56 and 5.18 mg/100g, respectively). In addition, it could be noticed that calcium, zinc, iron and manganese in fresh un-pollinated date were higher than doum fruit but doum fruit had the highest content of potassium and sodium (1626.72 and 396.12 mg/100g, respectively) (Table 2), so it may have useful for control of high blood pressure according to Aremu *et al.*, (2006). Manganese and zinc are essential minerals for preventing deficiency diseases and healthy for our bodies because the high content of zinc in date palm pollen is useful to manufacture of testosterone as essential for function of the male reproductive system and antioxidant according to Ali *et al.*, (2007).

Antioxidant compounds on (dry weight basis) and radical scavenging activity (DPPH%) on (fresh weight basis)

From data presented in Table (3), it can be seen that total phenolic compounds were the highest in fresh un-pollinated date (10.96 mg/g) followed by date palm pollen (4.96 mg/g) then doum fruit (4.63 mg/g). Phenolic compounds are played important roles as a class of antioxidant agents by chelating redox-active metal ions, preventing hydroperoxide conversion into reactive oxyradicals and inactivating oxygenase (Daoud *et al.,* 2015). Hydrolysable and condensed tannins of fresh un-pollinated date (1.50 and 1.76%, respectively) were higher than hydrolysable and condensed tannins of date palm pollen (0.27 and 1.10%, respectively) and doum fruit (0.59 and 0.93 %, respectively) (Table3). Tannins are known for their antioxidant activity by lipid peroxidation, free radical scavenging and inhibition of pro-oxidative enzymes (Koleckar *et al.,* 2008). Thus, antioxidant activity of fresh un-pollinated date (87.56%) was higher than date palm pollen (77.91%) and doum fruit (58.41%) that referring to tannins and phenolic compounds as a source of antioxidant.

Table 2. Minerals content of fresh un- pollinated Siwi date, date palm pollen and doum fruit (mg/100g on dry weight basis)

samples / minerals content	Fresh of un pollinated date	Date palm pollen	Doum fruit
Ca	167.16	491.82	129.97
Mg	109.58	185.61	143.69
K	720.49	1339.47	1626.72
Na	180.51	193.41	396.12
Zn	0.47	6.09	0.11
Fe	3.16	9.56	1.22
Mn	0.44	5.18	0.12

Table 3. Antioxidant compounds on (dry weight basis) and radical scavenging activity (DPPH%) on (fresh weight basis)

samples / compounds	Fresh un-pollinated date	Date palm pollen	Doum fruit	$LSD_{0.05}$
Total phenolic compounds (as gallic acid) (mg/g) (on DWB)	10.96 ± 0.525 [a]	4.96 ± 0.477 [b]	4.63 ± 0.553 [c]	1.0375
Hydrolysable tannins % (as tannic acid) (on DWB)	1.50 ± 0.04 [a]	0.27 ± 0.01 [c]	0.59± 0.02 [b]	0.0502
Condensed tannins % (as proanthocyanidin) (on DWB)	1.76 ± 0.03 [a]	1.10± 0.02 [b]	0.93 ± 0.04 [c]	0.0708
Radical scavenging activity %(on FWB)	87.56± 0.57 [a]	77.91± 0.22 [b]	58.41± 0.41 [c]	0.849

The mean value with different superscript alphabets in rows indicate significantly differences ($\rho \leq 0.05$) using LSD test
*** All items measured on dry weight basis (DWB) except radical scavenging activity which measured on fresh weight basis (FWB)**

HPLC analysis of phenolic compounds content in different methanolic extracts of fresh un-pollinated date, date palm pollen and doum fruit

From Table (4), results showed that fresh un-pollinated date, date palm pollen and doum fruit containing high quantities of phenolic compounds. The results revealed that all fractionated phenolic compounds in fresh un-pollinated date were higher than date palm pollen and doum fruit except caffeic acid and alpha-coumaric acid. Phenolic compounds of fresh un-pollinated date represented the highest value comparing with date palm pollen and doum fruit (Table 4). Also, caffeic acid and alpha-coumaric acid in date palm pollen were higher than fresh un-pollinated date and doum fruit (Table 4). Thus, the antioxidant activity of the phenolic components principally related to their redox properties which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Ramarathnam *et al.,* 1997).

Table 4. HPLC analysis of phenolic compounds content (mg/100g) in different methanolic extracts of fresh un-pollinated date, date palm pollen and doum fruit

Samples / Phenolic compounds	Fresh of unpollinated date	Date palm pollen	Doum fruit
Gallic acid	4.47	2.43	1.13
4- Amino benzoic acid	2.89	1.97	1.69
Protocatechuic acid	21.32	6.89	3.07
P-OH-benzoic acid	N.D	N.D	N.D
Chlorogenic acid	4.19	1.79	0.83
Vanillic acid	11.88	7.75	5.83
Caffeic acid	1.64	2.49	1.09
P-coumaric acid	5.59	4.35	1.42
Ferulic acid	8.79	4.61	3.09
Iso- ferulic acid	1.99	1.64	0.21
e-vanillic acid	37.28	15.65	8.12
Alpha-coumaric acid	2.34	2.65	0.70
Benzoic acid	25.98	N.D	6.39
Ellagic acid	9.35	5.34	3.38
3,4,5-methoxy-cinnamic acid	2.83	1.34	0.65
Cinnamic acid	0.49	0.27	0.13
Salycilic acid	5.85	2.79	2.12
Pyrogallol	17.60	14.84	10.88
Catechein	18.62	11.40	5.89
Catechol	16.36	4.03	1.66
Epicatechein	14.48	10.62	3.104
Caffeine	2.21	2.097	1.65
Coumarin	1.001	0.681	0.44
Reversetrol	0.89	0.59	0.37
Total	218.05	106.21	63.84

N.D: Not Detected

HPLC analysis of flavonoid compounds content (mg/100g) in different methanolic extracts of fresh un-pollinated Siwi date, date palm pollen and doum fruit

From data presented in Table (5), it is noticeable that flavoglucosides and flavonoids compounds of fresh un-pollinated date represented the highest value by comparing with date palm pollen and doum fruit. However, date palm pollen represented the highest value comparing with fresh un-pollinated date and doum fruit for quercetin-3-O-glucoside, apigenin-7-O-neohespiroside, naringenin, rhamnetin and apigenin (2.176, 0.929, 0.797, 0.912 and 1.696 mg/100g, respectively). These flavonoid compounds had an important role as antioxidant properties such as disconnection of radical reactions, scavenging free radicals and inhibition of oxidase enzymes (Nijveldt *et al.,* 2001).

Table 5. HPLC analysis of flavonoid compounds content (mg/100g) in different methanolic extracts of fresh un- pollinated Siwi date, date palm pollen and doum fruit

Sample / Flavonoid compounds	Fresh of un pollinated date	Date palm pollen	Doum fruit
Luteoline-6-arabinose-8-glucose	18.735	10.885	7.459
Luteoline -6-glucose-8-arabinose	8.698	1.737	0.608
Apigenin-6-arabinose-8-galactose	24.502	6.698	3.213
Apigenin -6-rhamnose-8-glucose	3.521	1.151	0.574
Apigenin -6-glucose-8-rhamnose	7.283	2.054	N.D
Luteoline-7-glucose	13.197	3.539	3.235
Quercetin-3-O-glucoside	N.D	2.176	N.D
Apigenin -7-O-neohespiroside	N.D	0.929	0.174
Kaempferol -3,7 dirhamoside	5.347	0.988	0.805
Acacetin neo-rutinoside	N.D	N.D	N.D
Kaempferol 3,2 p-coumaroyl glucose	9.029	6.530	N.D
Apigenin -7-O- glucose	1.196	0.819	0.204
Narengin	3.484	1.425	1.744
Hesperidin	19.027	9.274	2.443
Rutin	4.239	2.619	2.390
Quercetrin	3.591	1.793	1.733
Rosmarinic	0.935	0.372	0.627
Quercetin	2.544	2.524	1.666
Naringenin	0.208	0.797	0.165
Hesperitin	7.153	5.403	1.540
Kaempferol	0.312	0.188	0.087
Rhamnetin	0.704	0.912	0.174
Apigenin	1.398	1.696	0.373
Acacetin	8.866	1.664	1.472
Total	143.969	66.173	30.915

N.D: Not Detected

Cytotoxicity effect of fresh un-pollinated Siwi date, date palm pollen and doum fruit extracts against some of human cancer cells

From data presented in Table (6) and Figure (1), it can be seen that the effect of fresh un-pollinated date, date palm pollen and doum fruit extracts against colon cancer cells (HCT). It was found that IC_{50} (concentration able to decrease cells viability by 50% versus control cultures) of fresh un- pollinated date, date palm pollen and doum fruit extracts were 77, 90.7 and 220 µg/ml, respectively. Also, the best concentrations leading to the lowest percentage of survival fraction of colon cancer cells were 250 µg/ml in

fresh un-pollinated date extract (26.9%) and 500 µg/ml in date palm pollen and doum fruit extracts (48.4 and 40.0%, respectively). Previous, fresh un- pollinated date had the lowest percentage of survival cells at concentrations 250 and 500 µg/ml and it had the best concentration of IC_{50} against colon cancer cells. This effect related to antioxidant compounds which found in fresh un-pollinated date such as flavonoids and condensed tannins. These compounds had cytotoxicity agents. Thus, cytotoxicity agents will destroy the cancer cells which produced apoptotic bodies by arrest cell cycle and induce of apoptosis (Farshori *et al.,* 2003). From table (6) and figure (2), it is seen that IC_{50} of fresh un-pollinated date, date palm pollen and doum fruit extracts against prostate cancer cells (PC3) were 67.8, 90 and 361 µg/ml, respectively. Also, the best concentrations leading to the lowest percent of survival fraction of prostate cancer cells were 250 µg/ml in fresh un-pollinated date extract (15.0%) and 500 µg/ml in date palm pollen and doum fruit extracts (39.0 and 45.5%, respectively). Furthermore, fresh un-pollinated date extract had the best concentration of IC_{50} because fresh un-pollinated date contained a high amount of natural compounds such as condensed tannins which induce a different distribution of cells in the various phases of cells cycle with increasing in apoptotic cells. Also, these compounds were inhibition of cells growth of prostate cancer regardless of androgen receptor status according to Bawadi *et al.* (2005). By measuring the effect of fresh un-pollinated date, date palm pollen and doum fruit extracts against breast cancer cells line (MCF7) (Table 6) and (Figure 3), it is apparent that IC_{50} of fresh un- pollinated date, date palm pollen and doum fruit extracts were 83.1, 165 and 245 µg/ml, respectively. Also, the best concentrations leading to the lowest percentage of survival fraction of breast cancer cells were 250 µg/ml in fresh un-pollinated date and date palm pollen extracts (20.9 and 42.0%, respectively) and 500 µg/ml in doum fruit extract (48.2%). From previous results, fresh un-pollinated date extract had the lowest percentage at concentrations 250 and 500 µg/ml furthermore the best concentration of IC_{50}. This effect was related to compounds in fresh un-pollinated date with anti-proliferative activity such as flavonoids and tannins. From Table (6) and Figure (4), it was found that IC_{50} of fresh un-pollinated date and date palm pollen extracts against larynx cancer cells (HEP2) were 63.3 and 260 µg/ml, respectively. Also, the best concentrations leading to the lowest percent of survival fraction of larynx cancer cells were 125 µg/ml in fresh un-pollinated date extract (22.0%) and 500 µg/ml in date palm pollen and doum fruit extracts (41.6 and 53.2%, respectively). Previously, fresh un-pollinated date extract had the lowest percentage of survival cells at different concentration and it had the best concentration of IC_{50} because of natural compounds such as flavonoids which inhibited cells growth.

Table 6. Cytotoxicity effect of fresh un-pollinated Siwi date, date palm pollen and doum fruit extracts against some of human cancer cells

Sample	Surviving fraction of human carcinoma cell line (%)	Concentration (µg/ ml)					IC_{50} (µg/ ml)
		0	62.5	125	250	500	
Fresh of un-pollinated date	Surviving fraction of HCT	100.0	56.4	28.7	26.9	28.4	77
	Surviving fraction of PC3	100.0	52.6	26.4	15.0	20.6	67.8
	Surviving fraction of MCF7	100.0	56.7	37.0	20.9	24.8	83.1
	Surviving fraction of HEP2	100.0	50.0	22.0	22.5	26.7	63.3
Date palm pollen	Surviving fraction of HCT	100.0	54.6	44.8	51.1	48.4	90.7
	Surviving fraction of PC3	100.0	59.5	37.1	42.5	39.0	90
	Surviving fraction of MCF7	100.0	94.6	53.3	42.0	44.3	165
	Surviving fraction of HEP2	100.0	61.9	55.3	50.5	41.6	260
Doum fruit	Surviving fraction of HCT	100.0	58.1	55.1	48.2	40.0	220
	Surviving fraction of PC3	100.0	68.4	52.6	54.2	45.5	361
	Surviving fraction of MCF7	100.0	84.0	64.4	49.9	48.2	245
	Surviving fraction of HEP2	100.0	81.4	62.0	57.4	53.2	-

IC_{50} : Concentration able to decrease cell viability by 50% versus control cultures * HCT : Colon carcinoma cell line
*** MCF7 : Breast carcinoma cell line * PC3 : Prostate carcinoma cell line * HEP2 : Larynx carcinoma cell line**

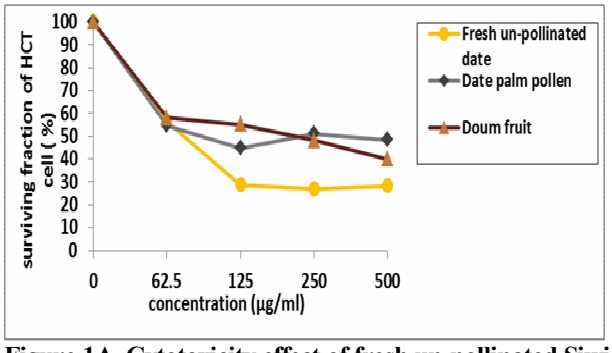

Figure 1A. Cytotoxicity effect of fresh un-pollinated Siwi date, date palm pollen and doum fruit extracts against human colon cancer cells

Effect of fresh un-pollinated date, date palm pollen and doum fruit extracts as antimicrobial agents

In the present study the antimicrobial activities of fresh un-pollinated date, date palm pollen and doum fruit extracts in different concentration (12.5, 25, 50 and 100 mg/ml) against some pathogenic microorganisms such as bacteria (*Escherichia coli, Pseudomonas aeuroginosa, Staphylococcus aureus* and *Bacillus subtilis*), yeast (*Candida Albicans*) and mold (*Asperigillus flavus*) were examined. The percentages of inhibition are correlated to the inhibition of bacteria by the antibiotic (Ampicillin) (1mg/ml), yeast and fungi with Clotrimazole (1mg/ml) (Table 7). The results indicated that all microbial were inhibited at different concentration except fresh un-pollinated date extract at concentration 12.5 mg/ml against gram positive bacteria *(Staphylococcus aureus* and *Bacillus subtilis*) and mold (*Candida Albicans*). Also, date palm pollen extract was not active at concentration 12.5 mg/ml against *Escherichia coli*. Also, doum fruit extract was not active at concentraction 25 mg/ml against all microorganisms except *Asperigillus flavus* and at concentration 12.5 mg/ml against all microorganisms. Fresh un-pollinated date had the best effect against gram

negative bacteria (*Escherichia coli* and *Pseudomonas aeuroginosa*) (Figures 5 and 6) and date palm pollen had the best effect against gram positive bacteria *(Staphylococcus aureus* and *Bacillus subtilis*), yeast (*Candida Albicans*) and mold (*Asperigillus flavus*) (Figures 7, 8, 9 and 10) (Table 7). Also, the best effect of fresh un-pollinated date extract against gram negative bacteria (*Escherichia coli* and *Pseudomonas aeuroginosa*) may be related to the highest amount of condensed and hydrolysable tannins in fresh un-pollinated date which played an important role as antimicrobial activity by inhibition of enzyme activity by complexation with substrates of bacteria and fungi; direct action of tannins on the microorganism metabolism, through the inhibition of oxidative phosphorylation; a mechanism involving the complexation of tannins with metabolic ions, decreasing the availability of essential ions to the metabolism of the microorganisms in agreement with Santos *et al.* (2009).

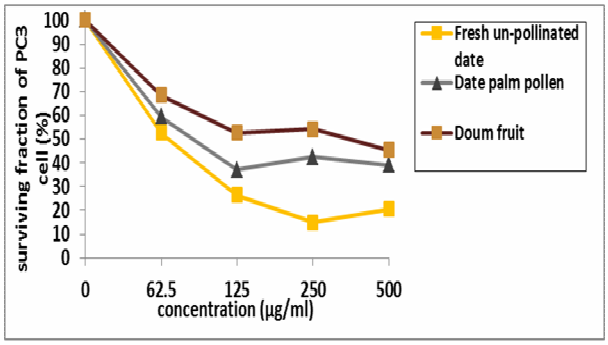

Figure 1B. Cytotoxicity effect of fresh un-pollinated Siwi date, date palm pollen and doum fruit extracts against human prostate cancer cells

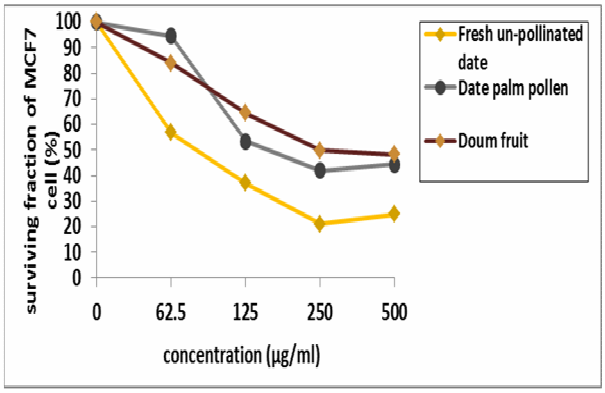

Figure 1C. Cytotoxicity effect of fresh un-pollinated Siwi date, date palm pollen and doum fruit extracts against human breast cancer cells

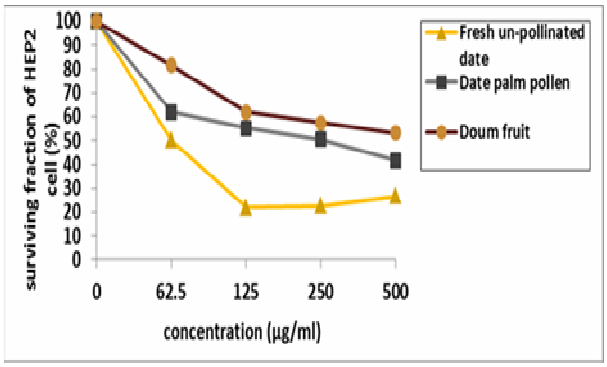

Figure 1D. Cytotoxicity effect of fresh un-pollinated Siwi date, date palm pollen and doum fruit extracts against human larynx cancer cells

Table 7. Effect of fresh un- pollinated Siwi date, date palm pollen and doum fruit extracts against some of microbial pathogenic

Samples	Conc mg/ml	*Escherichia coli ATCC 25922* mm	*Pseudomonas aeuroginosa CAICC 21* mm	*Staphylococcus aureus ATCC 29213* mm	*Bacillus subtilis CAICC 11* mm	*Candida Albicans CAICC 51* mm	*Asperigillus flavus CAICC 41* mm
Fresh of un-pollinated date	12.5	3.0 ± 0.2	4.1 ± 0.2	NO	NO	NO	2.2 ± 0.2
	25	6.0 ± 0.2	6.9 ± 0.3	5.1 ± 0.3	4.0 ± 0.2	5.2 ± 0.2	6.0 ± 0.3
	50	10.7± 0.3	10.0 ± 0.3	11.2 ± 0.2	9.9 ± 0.3	8.9 ± 0.3	8.1 ± 0.3
	100	12.9± 0.3	14.3 ± 0.4	16.0 ± 0.3	14.0 ± 0.3	11.1 ± 0.2	12.0± 0.3
Date palm pollen	12.5	NO	2.0 ± 0.1	5.9 ± 0.3	3.2 ± 0.1	1.8 ± 0.2	4.0 ± 0.3
	25	3.2 ± 0.2	4.9 ± 0.3	8.0 ± 0.2	7.0 ± 0.2	7.1 ± 0.1	8.9 ± 0.3
	50	7.0 ± 0.3	7.9 ± 0.3	13.0 ± 0.2	12.0 ± 0.2	11.3 ± 0.2	10.0± 0.2
	100	10.0± 0.2	11.0 ± 0.2	17.0 ± 0.2	14.9 ± 0.3	13.9 ± 0.3	13.0± 0.2
Doum fruit	12.5	NO	NO	NO	NO	NO	NO
	25	NO	NO	NO	NO	NO	2.0 ± 0.3
	50	3.3 ± 0.3	4.0 ± 0.2	6.7 ± 0.3	2.1 ± 0.3	3.9 ± 0.4	5.0 ± 0.2
	100	5.0 ± 0.1	6.9 ± 0.3	8.3 ± 0.3	5.0 ± 0.2	6.2 ± 0.3	6.9 ± 0.3
Ampicillin 1mg/mL		25	26	24	25	----	----
Clotrimazole 1mg/mL		----	----	----	----	26	23

Each value represent the mean value ± SD NO: Not Observed mm: Diameter of inhibition zone

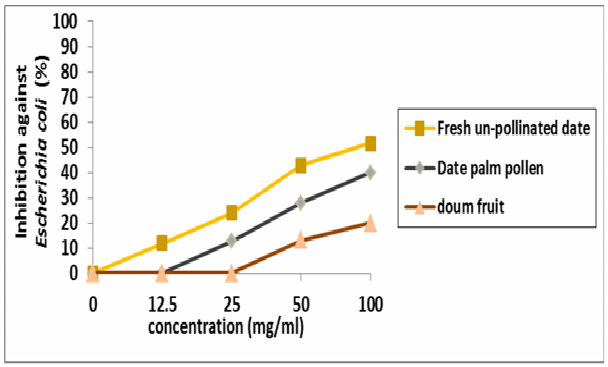

Figure 2A. Effect of extracts against *Escherichia coli*

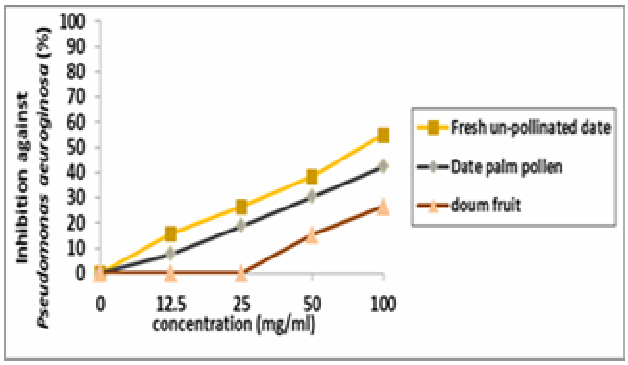

Figure 2B. Effect of extracts against *Pseudomonas aeuroginosa*

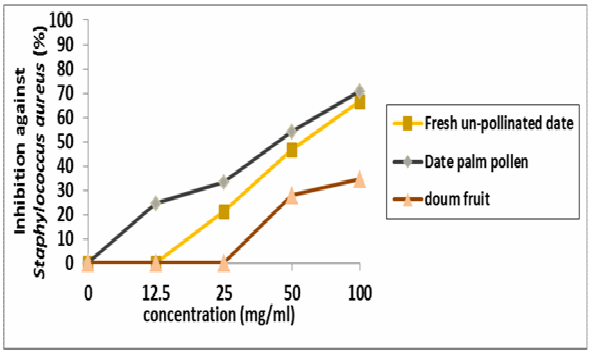

Figure 2C. Effect of extracts against *Staphylococcus aureus*

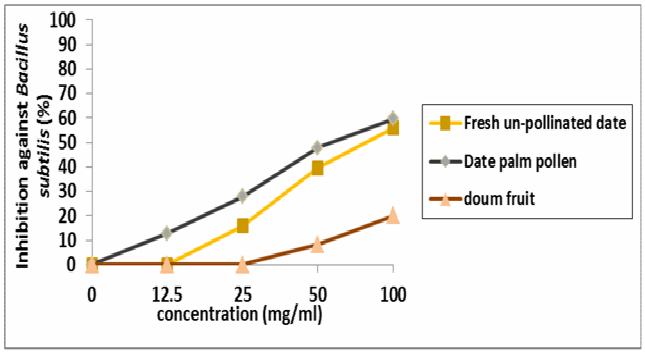

Figure 2D. Effect of extracts against *Bacillus subtili*

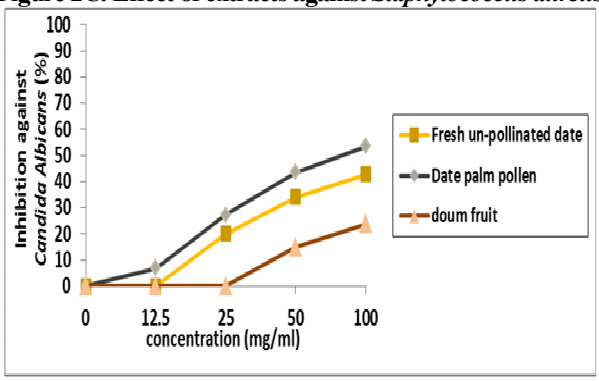

Figure 2E. Effect of extracts against *Candida albicans*

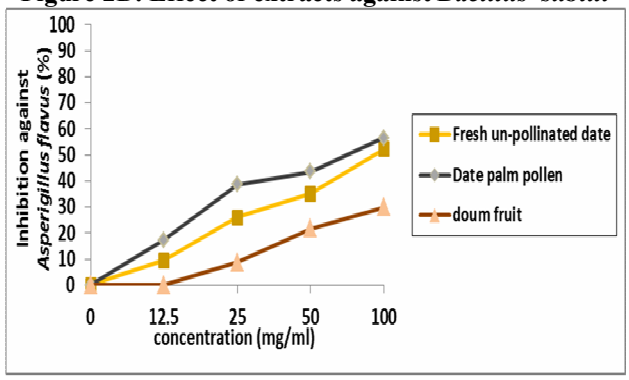

Figure 2F. Effect of extracts against *Asperigillus flavus*

CONCLUSION

The obtained data of the present study pointed that we neglected a very important bio-active product. This product is the un-pollinated date which considered as a byproduct of the date production. It is used as an animal feeder. However, it contains antioxidant compounds and its main effect as anticancer and antimicrobial. So, we recommended to use the un-pollinated date as a human food or as food additives.

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دراسات على التأثير البيولوجي لبعض الأغذية المنتقاه (البلح السيوي الغير مخصب الطازج و طلع النخيل و فاكهة الدوم)

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الهدف من هذه الدراسة هو دراسة التأثير البيولوجي للبلح السيوي الغير مخصب الطازج و طلع النخيل والدوم ضد بعض الخلايا السرطانية و بعض الميكروبات الممرضة كما تم تقدير التحليل الكيماوي لهذه الأغذية والذي يشمل الرطوبة و المواد الصلبة الذائبة و الرماد والسكريات الكلية والمختزلة وتقدير بعض المركبات الفعالة المضادة للأكسدة كالفينولات الكلية والتانينات الذائبة و المكثفة وكذلك القدرة على مسك الشقوق الحرة وأيضا تم عمل تفريد للمركبات الفينولية و الفلافونيدات وتقدير تأثيرات البيولوجية لمستخلصات البلح السيوي الغير مخصب و طلع النخيل و الدوم ضد بعض الخلايا السرطانية في القولون و البروستاتا و الثدي و الحنجرة وبالإضافة إلى تأثيرهذه المستخلصات المضاد لبعض الكائنات الحية الدقيقة الممرضة كالبكتريا و الفطر و الخميرة . أظهرت النتائج إرتفاع محتوى البلح السيوي الغير مخصب من المركبات المضادة للأكسدة مقارنة بطلع النخيل و الدوم كالفينولات و التانينات الذائبة و المتكثفة و الفلافونويدات و بالتالي يؤدي إلى قدرة عالية على مسك الشقوق الحرة. على الجانب الأخر وجد أن طلع النخيل الأعلى في محتواه من بعض العناصر المعدنية كالكالسيوم و الماغنسيوم و الحديد و الزنك و المنجنيز مقارنة بالبلح السيوي الغير مخصب و فاكهة الدوم كما وجد أن أفضل تأثير مضاد على الأربع الخلايا السرطانية المختلفة في العينات هو تأثير البلح الغير مخصب الطازج حيث موت الخلايا السرطانية يزيد عن 50% عند إستخدام مستخلص البلح الغير مخصب بتركيز 62 ميكروجرام/مل تقريبا لكن أفضل إنخفاض للخلايا السرطانية الحية يصل إلى 25% تقريبا عند إرتفاع تركيزات المستخلصات إلى 125 و 250 ميكروجرام/مل. كما وجد أن البلح الغير مخصب أفضل تأثير مضاد للميكروبات السالبة للجرام لكن كان تأثير طلع النخيل الأفضل ضد الميكروبات الموجبة للجرام والفطر والخميرة وبذلك أوضحت هذه الدراسة أننا نهمل أهم المنتجات الحيوية.

الكلمات الإفتتاحية: الخلايا السرطانية – الكائنات الحية الدقيقة الممرضة – المركبات المضادة للأكسدة – القدرة على مسك الشقوق الحرة