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Antioxidants Activity of Date Seed Extraction of Some Date Varieties

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Abstract

The study included preparing aqueous , alcoholic, and oil extractions of date seed power of (*Phoenix dactylifera*) Al Hillawi, Al Khadrawi and Al ZAHDY date varieties estimating its total contents of phenol and flavonoids, and then it is studied its antioxidants activity, reduction strength and ability to bon Ferrous ion. The results showed that total contents of phenolic compounds are ranged from 56.16 to 67.32 mg/ml for the ethanol extraction, 56.6 to 65.6 to 65.32 mg/ml for methanolic extract and 27.88 to 40.06 mg/ml for aqueous extraction. While the total contents of flavonoids of aqueous extract is ranged from 22.12. to 33.32 mg/ml, 40.21 to 52.16 mg/ml in the ethanolic extract and from 35.11 to 46.16 mg/ml in the methanol extraction. Indeed, aqueous and alcoholic extract of (*Phoenix dactylifera*) Al Hillawi, AlKhadrawai and Al Zahdy give Antioxidation activity are ranged from 37.50% to 88.70%, reduction strength is ranged from 0.895 to 2.63 and the value of Ferrous ion bon is ranged from 41.92% to 60.93%.

Keywords: Date seed, antioxidants and phenolic compounds

Introduction

Dates seeds are deemed of the most important waste of date industry representing very important economical resources and at same time may cause environmental problem if it is accumulated considerably in the nature²². The new studies reported that dates seeds have high level of phenolic compounds, Flavonoids, antioxidants, Dietary fibers higher than those are reported in flesh part. there are high levels of α -Tocopherol, Ascorbic acid and Glutathione and of polyphenol compounds such as Sinapic acid , Caffeic acid with quantities of protocatechnic acid¹¹, also it is diagnosed multi-aromatic in dates seeds includes alcohols, citrates, aldehydes, Ketones, saturated and unsaturated hydrocarbonates¹⁵. The study discussed Phenolic , Flavonoids contents and Antioxidant of date seed extracts, it is also observed that Dietary fiber extracted from date seed are Polyphenoles-riche¹⁹.⁹ suggested that the date seed contents in Phenolic compounds are ranged from 21.0 to 62.0 mg Gallic acid/100g . In addition to studies that was made to extract oil from date seed and use it in food, Pharmaceutical, Plastic industries¹⁰. Whereas date seed is rich source of important components such as oil, protein, dietary fibre, carbohydrate are deemed source of phenolic compounds and antioxidants; and to exploit these materials which respecting a valuable by-

product of date fruit processing industries and there was no local study approach this matter in detail; therefore, we found that it is necessary to perform this study to extract bioactive compounds from date seed powder and to estimate of Phenolic and Flavonoids compounds and their antioxidant activity.

Method and Materials

(*Phoenix dactylifera*) Halawi , Khadrawi , Zahdi date seed were obtained from Local market , Basrah . Separate seed from date , then their wishes , drier and milling to smooth powder then care in sterilize and close cups to uses then .

2-Preparation of Date seeds Extract s

A- Aqueous Extract

By applying method of¹⁴, be weighed 20g from date seed powder and their add 500ml of distilled aqueous , drop out in Shaking incubator for 24 hours at 28C° then centrifuged at 2500 Round/min for ten minutes , then filtration by Whitman No1, concentrated of filtrate by Rotary Vacuum Evaporator to thick liquid then placed in incubator at 37C° for 48 hours get at dry powder of extract , take care in showdown and obscured glasses package and cured in refrigerator at 4C°.

B- Ethanolic Extract

Ethanolic extract were prepared by weighting 100g of date seed powder dissolved in 500ml ethanol (98%) and mixed well , left for 24hours at laboratory temperature (25-30C°), filtration by Whatman No1 , concentrated by Rotary Vacuum Evaporator at 40C° and left at laboratory temperature to get at dry powder , take care in showdown and obscured glasses package and cured in refrigerator at 4C°⁸ . .

C- Methanolic Extract

Two steps applied in paragraph A to extract Methanolic date seed powder.

D- Oil Extracts

It is extracted date seed oil from seed powder to study variety according to⁵ using mixture of aqueous , methanol and chloroform solvents.

Determination of total phenols*

It is estimated total phenolic compounds in date seed extracts by using (Folin –Ciocalteu) received in²¹, and uses Standard curve from Gallic acid in concentration between 10 to 200 mg/ml to calculating phenolic amount in extracts depending the scatter graph between acid concentration and absorption at 760 nanometer .

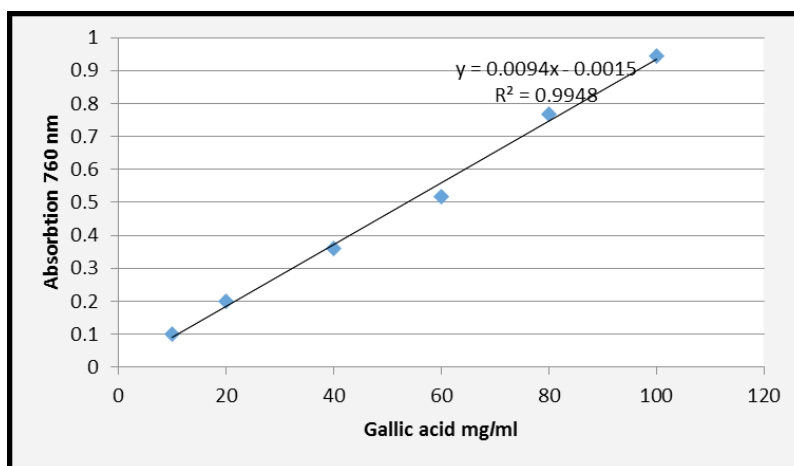
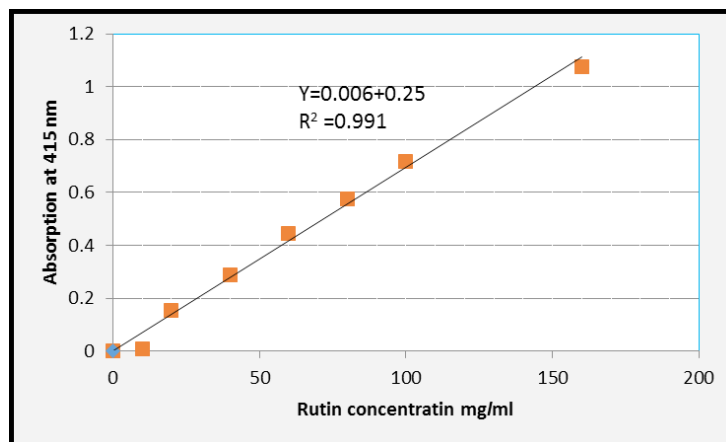


Fig 1: Standard curve of Gallic acid

*** Determination of total Flavonoids**

It was solved of 1gm from date seed extract in 1.5 ml ethanol, add it Aluminium chloride $AlCl_3 \cdot 6H_2O$ (2%) this perpetrated in 100ml methanol , mixed well, after 10 minutes calculate Absorption at 367 nanometer, and preparation to concentration of Flavonoid compound (Rutin) from 0 to 100 mg/ml, then determination of Flavonoids depending on correlation between concentration of rutin standard solution and Absorption at 415 nm.



.Fig 2: Standard curve of Rutin

Antioxidant activity

1-Scavenging of free radical ability

it is assessed ability to date seed extracts for scavenging of free radical according to ¹⁷, and used the equation for calculate activity:

$$\text{Scavenging of free radical \%} = \left[1 - \frac{\text{Sample absorption}}{\text{Control absorption}} \right] \times 100$$

2-Reducing power

It is applied the method by ¹³, the increase of absorption to mixture interaction it was indicator stating high reduction power.

3-Chelating of ferrous ion

Followed the method Decker and Welch (1990), calculated of chelating ability by next equation :

$$\text{Chelating of ferrous ion \%} = \left[1 - \frac{\text{Sample absorption}}{\text{Control absorption}} \right] \times 100$$

Results and Discussion

1-Total Phenolic Content

Results of the total phenolic compounds on Halawi, Khadrawi, Zahdi date seed is presented in Figure 6 below, the result of Statically analysis showed presence Significant differences (P<0.05) in phenolic contents by difference solvent extracts and seed source, Alcoholic extracts (Ethanolic and Methanolic) exhibited the highest content of phenolic compound compared with aqueous extracts .

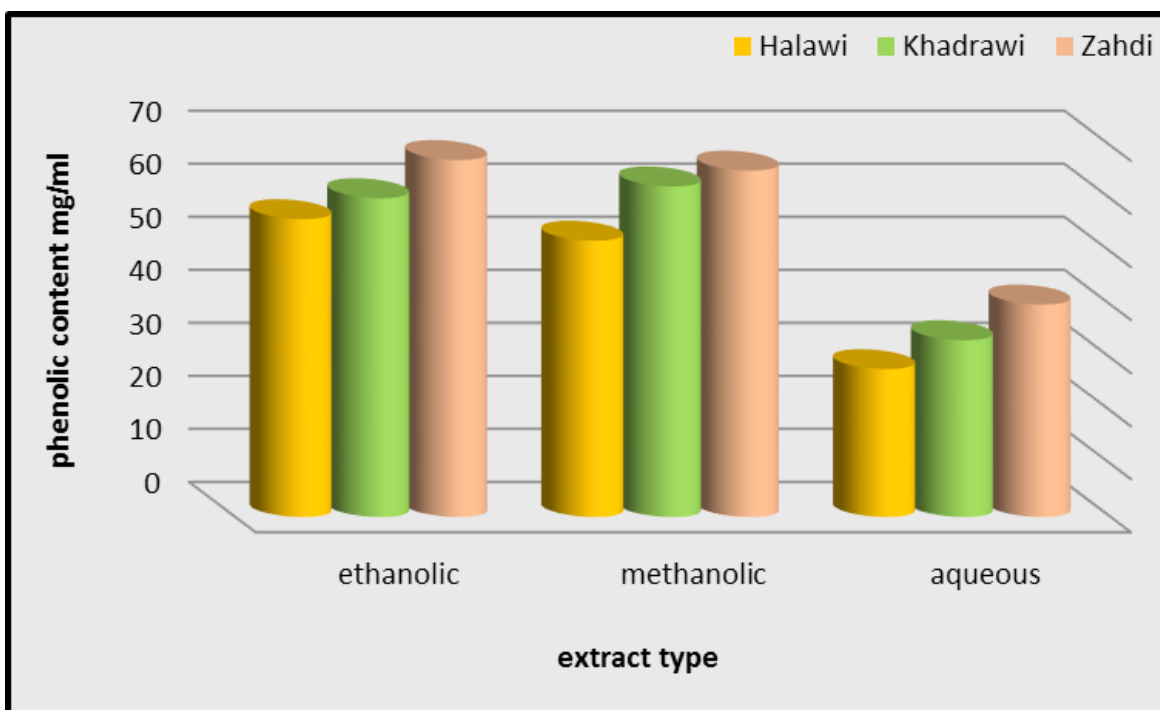


Fig 3: Total phenolic content of date seed extracts

The results is in agreement with ⁶ and ¹⁸ as it is found that e phenolic content in aqueous extract lower than ethanolic date seed extract, and the Statically analytic showed Significantly affected ($P<0.05$), the higher content of phenolics in Zahdi date seed that all extracts reach 67.32 , 65.32 , 40.06 mg/ml for ethanolic , methanolic, aqueous respectively, and then Khadrawi 60.11 , 62.33 , 33.34 mg/ml for 3 extracts respectively and the lower content of phenolic compound was Halawi date seed 56.16 , 52.11 , 27.88 mg/ml respectively, as it is agreed with same results of ⁶.

2-Total Flavonoids content

Figure 7 clarified Flavonoids content in date seed extracts as the result showed that there is important differences between aqueous and alcoholic extracts ($P<0.05$), ethanolic extract of Zahdi seed excellence in Flavonoids reach to 52.16 mg/ml and ethanolic extract of Khadrawi 50.11 mg/ml then ethanolic extract of Halawi seed 40.21 mg/ml , followed methanolic extracts , Halawi extract was the lowest in flavonoids 35.01 mg/ml , then Khadrawi 40.83 mg/ml and methanolic extract of Zahdi reach to 46.16 mg/ml , then aqueous extracts 22.12, 27.00 , 33.32 mg/ml of Halawi ,Khadrawi , Zahdi date seed respectively, the central reason in differences of Flavonoids return to growth condition, maturity stage,

geographic location , manuring method , soil disease types, storage conditions and extracts system ⁴ . .

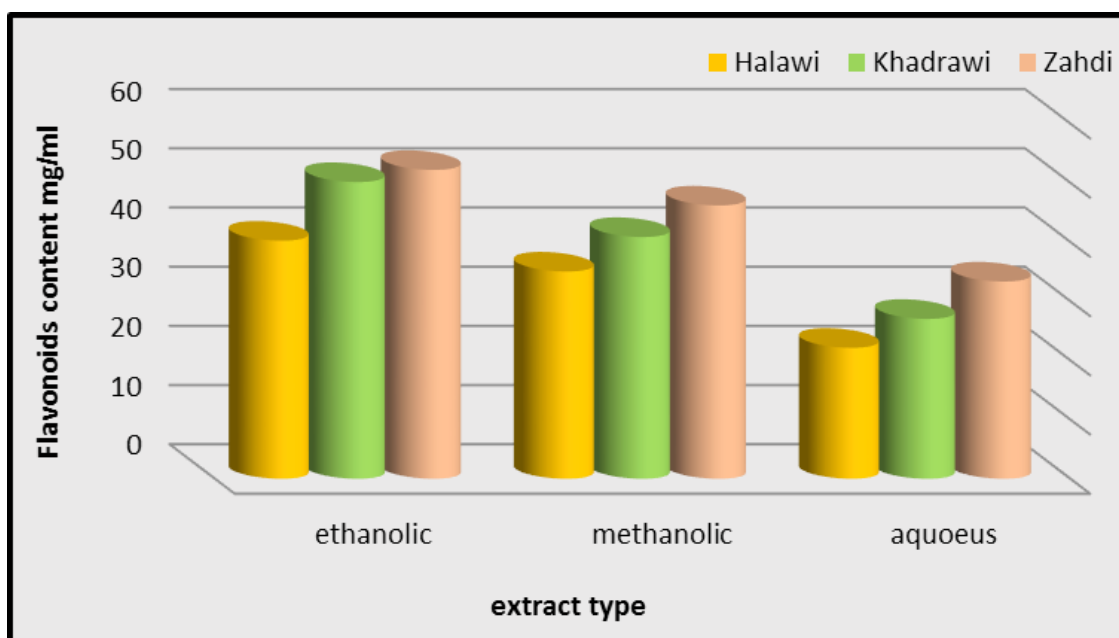


Fig 4: Total Flavonoids content of date seed extracts

Antioxidant activity of date seed extract

It is determined antioxidant activity by measure of date seed ability to free radical scavenging by uses(DPPH reagent) Diphenyl-1-Picrylhydrazyl compared to(BHT) Butylated Hydroxy Toluene , explain to Figure 8 A,B,C , the result of Statical analysis showed Significantly differences ($P<0.05$) of antioxidant activity to extracts as difference of seed source , and there were noticed the antioxidant activity increasing when concentration increased for all extracts , As Halawi

date seed (A) the methanolic extract showed the higher antioxidant 83.20% at 1.25 mg lml , when antioxidant activity of BHT 88% as the same concentration , then oil extract of antioxidant activity reach to 83.10% and ethanolic extract 61.2% , while the aqueous extract reach to 37.5% as the same concentration. The antioxidant activity of Halawi date seed differs at solvent extract of differences , it was posses the highest of scavenging of free radical DPPH compared to BHT , this due to presence bioactive compounds these posses antioxidant activity

to responsible to termination oxidation reaction by presence hydroxyl group donate hydrogen and reaction with free radical their convert to stable products, and the result is shown in Figure B antioxidant activity (DPPH) to Kadrawi date seed extracts, when ethanolic extract give a higher activity than methanolic, oil and aqueous reach 88% at concentration 1.25mg/ml it was equal BHT activity 88%, these results is in agreement with¹² when they found ethanolic extract of date seed give a higher antioxidant when uses DPPH radical as compared with aqueous extract, that return to a high content of phenolic compound and flavonoids which posses scavenging free radical due to presence hydroxyl group which donate electron to free radical, then oil extract of Khadrawi seed reach 85.2% and methanol extract 85%, while aqueous extract of Khadrawi seed gives the lowest antioxidant reach 42.4% as the same concentration because of a little content of phenolic compounds due to a limit ability of water to extract phenolics 162g/kg and flavonoids 17 g/kg from date seed due to a low solubility to these compounds in water², in addition of the uses of water alone induce to extraction other compounds such as protein, polysaccharides and induce to appearance inclusions through filtration and hardness to disposed³.

Reducing power

The result of statical analytic showed height significantly ($P < 0.05$) in reducing power values as increasing of extracts concentration to all types of seed, in Figure A4 Halawi date seed gives clear reducing power compared with α -tocopherol which reach to 2.689, and noticed the methanolic extract appear the higher reducing power compared with other extracts through Absorbance values when increase Absorbance that mean a higher reducing power reach to 0.864 for methanolic extract as concentration 0.25 mg/ml and increase when concentration increasing reach to 2.527 at 1.25mg/ml, while ethanolic extract reach to 0.4, 0.7, 1.09, 1.513 and 1.733 at concentrations 0.25, 0.5, 0.75, 1 and 1.25mg/ml respectively when reducing power reach to 0.762, 0.822, 0.94, 1.223 and 1.552 for oil extract at concentration 0.25, 0.5, 0.75, 1, 1.25 mg/ml respectively. were aqueous extract appeared the lowest reducing power ranged from 0.362 to 0.895 when concentration increase from 0.25 to 1.25 mg/ml. Also, the result showed in Figure 9 B reducing power of Khadrawi date seed, ethanolic extract appeared the higher reducing power compared with methanolic, oil and aqueous reach to 2.650, while reducing power for other extract reach to 0.8, 1.223, 1.534, 1.923 and 2.223

for methanolic extract at concentration 0.25, 0.5, 0.75, 1, 1.25 mg/ml, and 0.223, 0.433, 0.622, 0.792 and 0.834 for aqueous extract and 0.752, 0.866, 0.993, 1.523 and 1.690 for oil extract as the same concentration, in Figure C noticed reducing power for ethanolic extract of Zahdi date seed 2.632 equal reducing power α -tocopherol at concentration 1.25 mg/ml followed methanolic extract reach to 2.342 then oil extract reach to 0.821, 0.892, 1.003, 1.45, 1.732 at concentration 0.25, 0.5, 0.75, 1, 1.25 mg/ml, and that were the lowest reducing power for aqueous extract reach to 0.432, 0.634, 0.793, 0.875 and 0.993 at concentration 0.25, 0.5, 0.75, 1 and 1.25 mg/ml.

Chelating of ferrous ion

The percentage of date seed extracts ability to chelating of ferrous ion comared with Ethylene Di-amine Tetra acetic acid Di-sodium, the statical analytic showed significantly differences ($P < 0.05$) among prepared extracts of chelating ability, in Figure A that the higher ability to chelating of ferrous ion were ethanolic and oil of Halawi date seed reach to 53.56% and 51.22% at concentration 1.25 mg/ml respectively then methanolic extract and aqueous extract, while Khadrawi date seed (B) the ethanolic extract appeared a higher chelating than previous extract 59.32% and lower than ethanolic extract of Zahdi date seed 60.93% at concentration 1.25mg/ml, while oil extract reach to 56.00% for Khadrawi, followed methanolic extract that appeared chelating ability reach to 48.03% then aqueous 42.97%, also ethanolic and methanolic of Zahdi date seed appeared chelating ability (C) a highest compared with previous two types reach to 60.93% at concentration 1.25% followed oil extract 52.75% then methanolic extract 52.23%, this values were less compared with EDTA-2Na reach to 92.00%, while aqueous extract appeared less ability 41.92% at concentration 1.25mg/ml, this results agree with¹² were found that aqueous extract chelating ability was less than ethanolic extract at concentration 20% and the chelating of ferrous ion return to phenolic compounds that a manner role of chelating ions that oxidation catalyst such as iron and copper¹⁶.

Conclusion

Capability to benefit from date manufacturer by product to produce natural antioxidant alternative to artificial antioxidant that highly effective side. Alcoholic extracts give the higher of phenolic and flavonoids content than aqueous extract. Alcoholic extracts posses

the higher antioxidant than aqueous extracts while the antioxidant of oil extracts higher than aqueous extracts

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under College of Agriculture, University of Basrah, Basrah, Iraq and all experiments were carried out in accordance with approved guidelines.

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