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A Relative *in Vitro* Evaluation of Antioxidant Potential Profile of extracts from Pits of *Phoenix dactylifera* L. (Ajwa and Zahedi Dates)

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***Abstract*—**
Objective: A comparative study to evaluate the antioxidant and radical scavenging potential of pits of *Ajwa* and *Zahedi* varieties of *Phoenix dactylifera* L.
Method: Fresh *Ajwa* dates were bought from Madinah while *Zahedi* dates were from local market of Lahore. Dried and ground pits were primarily extracted in different solvents like methanol, acetone, n-hexane, chloroform, n-butanol and aqueous successively. Radical scavenging capacity and antioxidant potential of all extracts were assessed by using total phenolic contents (TPC) and total flavonoid contents (TFC), DPPH and $ABTS^{+}$ radical scavenging, lipid peroxidation inhibition activity, reducing power assay and phosphomolybdate assay.
Result: Results showed that the methanolic extract of *Ajwa* had the highest values; 74.19 ± 0.026 µg/mL of equivalent of Gallic acid. The aqueous extract of *Ajwa* pits has highest rutin equivalent and the potential to have significant antioxidant activity. Acetonic extract of both varieties had a prominent scavenging effect in DPPH and lipid peroxidation assay. Aqueous extract of *Ajwa* pits showed better ABTS radical scavenging capability (26.90 %) whereas the acetonic extract of *Zahedi* pits came close to it (26.02 %). TEAC values expressed in mM exposed the same results. Methanolic, acetonic and aqueous extracts showed effective antioxidant activity.
Conclusion: Date stones possess antioxidant properties and hence, are beneficial. Both varieties have substantial antioxidant potential. Epidemiological manifesto proposes an inverse relationship between dietary intake of flavonoids and cardiovascular risk. Cumulate evidence from our experimental studies indicates significance flavonoid content in pits of *Ajwa*, which might lower the risk of cardiovascular diseases. Therefore it is suggested that the crude methanol and aqueous extracts of pits need to be explored further for *in vivo* investigations to exploit novel anticardiovascular drugs.
***Index Terms: Ajwa, Zahedi, Phoenix dactylifera* L., pits, antioxidant, phenolics, flavonoids.**

I. INTRODUCTION

From the ancient time, fruits and vegetables are estimated as an essential gear of human diet. High intake of fruits and vegetables decreases risk of several diseases. Increased fruit and vegetable consumption is associated with a decreased incidence of cardiovascular diseases, cancer, and other chronic diseases. The beneficial health effects of fruits and vegetables have been attributed, in part, to antioxidant flavonoids present in these foods [1]. The trend of using various parts of plants in medicinal industry for the ailment of diseases now drifts from allopathic medicines to herbal resources owing to the side effects of synthetic drugs [2]. Because of antioxidant property and nutritional values, fruits have expanded special importance to nutritionists and other natural products chemists as panacea. All dates varieties growing in arid area share one botanical nomenclature; *Phoenix dactylifera* L. (genus *Phoenix* and family *Palmacae*) owing to its marvelous diversity [3]. There are more than 400 kinds of dates

under the name of *Phoenix dactylifera* L. [4].Among 6 species, only *Phoenix dactylifera* L. provides comestible fruit.

Phoenix dactylifera L. has eminent pharmacological importance, it works against skin disease, exhibits anti-microbial properties, reduces intestinal pain, effective against inflammation of the kidney. Nourishing constituent in dates helps to enhance the immune system by giving strength to the body hence suggested to break the fast with dates. Furthermore, it reduces labor pain and is recommendable for expecting women [5]. *Zahedi* date which is golden brown in hue originates from Iran, while *Ajwa*, an emerging plant in pharmacological research, can be grown only in Madinah, K.S.A. *Ajwa* date has all qualities of a date but is distinguished of its use as an antidote [6], anti-inflammatory and suppresser for cardiac diseases [7].

The present study focuses on the comparative antioxidant profiles of pits of *Ajwa and Zahedi* dates at their fully ripped stage *(Tamur* stage*).*

II. MATERIAL AND METHODS USED

A. Chemicals and Equipments

Methanol, chloroform, ethanol, n-hexane, acetone, Gallic acid, hydrochloric acid sulfuric acid, aluminum chloride, ammonium molybdate, Iron (III) chloride. hexa hydrate, mono potassium phosphate, ammonium thiocyanate, FC Reagent, Iron (II) chloride. tetra hydrate, di-potassium phosphate, potassium persulfate, potassium ferricyanide, potassium chloride, sodium nitrite, sodium acetate, sodium chloride, sodium chloride, sodium hydroxide and disodium hydrogen phosphate were bought from Riedel deHaën, Germany, Ascorbic acid (Fisher Scientific, UK), Tween 20 (Fisher Scientific), Linoleic acid (BioPLUS fine research chemicals), n-Butanol (Merck, Germany), Rutin (Alfa Aesar GmbH & Co. Germany), Trichloroacetic acid (Uni-Chem Chemical Reagents, USA). Trolox, BHA, Glacial acetic acid, ABTS, DPPH radical were bought from Sigma-Aldrich. Absorbance readings were taken using UVD-3200 UV-vis spectrophotometer (Labomed, Inc., USA). All the chemicals and solvent used in the experiment were of analytical grade.

B. Plant Collection

Ajwa dates were collected from the food markets of Al Madinah - Al Monawarah, Saudi Arabia and *Zahedi* from Lahore, Pakistan respectively and refrigerated. Pits from dates were manually separated and washed with double distilled water. Samples were shadow dried (for 4 days), and the dried pits of both varieties were pulverized separately into fine powders for further use.

C. Preparation of Extracts

The dried pit powder of each variety was extracted using six solvents which were carefully chosen on the basis of variable polarity; methanol, acetone, n-hexane, chloroform, n-butanol and aqueous medium. 150 grams of dried sample were extracted against five solvents using Soxhlet apparatus and concentrated using Rotavap (Fisher Scientific). Aqueous extract was prepared by adding 150 grams of sample to 500 mL of distilled water at 40 °C with continuous stirring for 48 hours. Solution was filtered and concentrated. Later, all the six extracts and the fractions were subjected to different antioxidant and radical scavenging assays. All the measurements were taken in triplicate and the data are given as mean ±SEM for three determinations.

D. Antioxidant and Radical Scavenging Assays

I. Total Phenolic Content (TPC) Assay

TPC was estimated by Folin-Ciocalteu method using Singleton and Rossi (1965) assay [8]. A mixture solution of 40.0 µL from plant extract solution (3.0 mg/mL) and 3.16 mL of distilled water was oxidized with the addition of 200 µL of FCR. To this solution, 600 µL of sodium carbonate solution was added after 8 minutes to neutralize the mixture. The mixture was incubated for 30 minutes at 40°C and absorbance was recorded at 765 nm using double beam spectrophotometer (UV-1800, Shimadzu).

For positive control, various dilution of gallic acid was used to give standard calibration curve. TPC of Phoenix dactylifera L. varieties were expressed as µg/mL of GAE (Gallic Acid equivalent). For negative control, methanol (solvent) was used in place of sample.

II. Total Flavonoid Content (TFC) Assay

TFC was determined by following the method reported by Park et al., 2008 [9]. 300 µL of sample extract (3mg/10mL) was diluted with 3.4 mL of aqueous methanol and oxidized with 150 µL of 0.5 M $NaNO_2$ solution. To this, 150 µL of 0.3 M $AlCl_3$ solution (0.725 grams of $AlCl_3.6H_2O$ in 100 mL) and 1.0 mL 1.0 M NaOH solution were added with 5 minutes interval before each addition and mixed prudently. For positive control, various dilution of rutin was used instead of plant extract to give standard calibration curve. Negative control was prepared by using methanol instead of sample. Absorbance was recorded at 506 nm. TFC of *Phoenix dactylifera* L. varieties were expressed as µg/mL of rutin equivalent.

III. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Assay

DPPH assay was used to evaluate the antiradical activity of *Phoenix dactylifera* L. following the method of Brand-Williams *et al.*,1995 [10]. Stock solution of DPPH (0.012g DPPH/ 50 mL of methanol) was prepared in dark. From stock solution, working solution was made by adjusting its absorbance to 0.97 (±0.03) at 516 nm. 100 µL of sample solution (1 mg/mL) was mixed thoroughly with 3.0 mL working solution of DPPH and incubated for 30 minutes at 37°C. Then absorbance was measured at 516 nm. Similarly, positive control was prepared by adding Gallic acid solution instead of extract solution in 3mL of DPPH solution. Negative control was recorded by adding 100 µL solvent used (methanol) in 3.0 mL DPPH working solution. Percentage inhibition was calculated according to given equation:

Inhibition (%) = ($Abs_{control}$ - Abs_{sample})/$Abs_{control}$ x100

EC_{50} is the efficient concentration at which sample inhibits 50 % of DPPH free radicals. It was evaluated after observing inhibition at concentration of 0.001g/mL.

IV. 2,2'-Azinobis (3-Ethylbenzo Thiazoline)-6 Sulphonic acid cation {ABTS}Assay

ABTS decolorization assay was assessed by following the protocol developed by Re et al., 1999 [11]. Stock solution 0.007 M of ABTS solution (0.03836g/9.5 mL water) was prepared and oxidized by adding 245 µL of potassium persulfate solution (100 mM) to dark blue green color solution. Ultimate volume was made up to 10mL. The solution was placed in dark, for 18 hours at room temperature to activate ABTS radical cation. The absorbance of stock solution was adjusted to 0.70 (±0.02) at 730 nm with 0.1 M phosphate buffer saline, pH 7.4.2.99 mL of ABTS working solution was taken in a cuvette. To this, 10 µL of extract solution (or standard solution) was added, mixed and its absorbance for 8 minutes, after every 30 seconds. Similarly, negative was recorded by adding solvent in place of extract solution. Percentage inhibition was calculated by given equation:

Inhibition % = [(Abs_{blank} – $Abs_{extract}$)/Abs_{blank}] x 100

Where **Abs_{blank}** is the absorbance of solution containing solvent only and **$Abs_{extract}$** is the absorbance of extract solution.

Antioxidant activity was also being expressed as trolox equivalent antioxidant capacity (TEAC) in mM

V. Reducing Power Assay

Reducing power assay was conducted by method of Oyaziu et al., 1986 [12]. 2.5 mL of extract solution (1 mg/mL) was mixed with 2.5 mL of 0.2M sodium phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide solution (1 %). The mixture was incubated at 50 °C for 20 minutes. To the mixture after incubation, 2.5 mL of 10 % trichloroacetic acid solution was added and green mixture was centrifuged at 6000 rpm for 10 minutes. 5.0 mL of its supernatant was diluted with 5.0 mL of distilled water with the addition of 1.0 mL of 0.1 % ferric chloride solution and mixed well. Then mixture was allowed to stand for 10 minutes. Absorbance was recorded at 700 nm. For negative and positive control, 2.5 mL of solvent and Gallic acid solution were added respectively in place of extract solution. The reducing power was estimated directly in Absorbance. Higher the absorbance, higher will be the reducing ability.

VI. Phosphomolybdate Assay

Phosphomolybdate assay, reported by Chatterjee et al., 2008 [13] was followed to quantify the reducing capacity of the samples under study. 300 µL of plant extract (3mg/ 10 mL methanol) was mixed with 3.0 mL of phosphomolybdate reagent which was prepared by mixing 100 mL of 0.6 M H_2SO_4 solution, 100mL of 0.004 M ammonium molybdate solution and 100 mL of 0.028 M Sodium phosphate solution. The samples were incubated at 95 °C in water bath for 90 minutes in dark. On cooling, absorbance was recorded at 765 nm. Antioxidant capacity was expressed in ascorbic acid equivalent (µg/mL of AAE). For negative and positive control, methanol and ascorbic acid solution were added respectively instead of extract solution.

VII. Lipid Peroxidation Assay

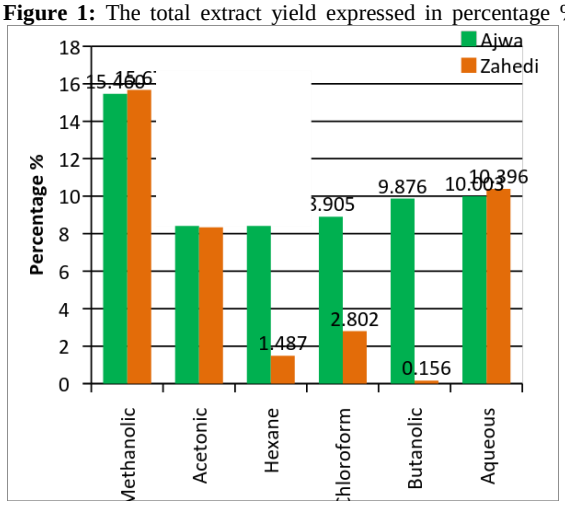
Lipid peroxidation assay was done by procedure reported by Keser et al., 2012 [14]. 100 µL of plant extract or butylated hydroxyanisole (BHA) solution (5 mg in 1 mL methanol) was diluted with 2.4 mL of 0.04 M phosphate buffer (pH 7.0), and mixed with 2.5 mL of linoleic acid emulsion (175 µL Tween-20 + 155 µL linoleic acid in phosphate buffer) and volume was made up to 50 mL with water. The mixture was placed in incubator at 37° C for 25 minutes. 100 µL of this mixture was taken after every 24 hour intervals and dissolved with 3.7 mL ethanol. It was reacted with 100 µL of 0.02 M ferrous chloride first and then with 100 µL of 30 % ammonium thiocyanate solution. Pink to red color would be observed. Blank contain only 2.5 mL emulsion and 2.5 mL buffer only. Then absorbance was recorded at 500 nm. Percentage Inhibition was calculated by following equation:

Inhibition (%) = (Abs_{blank} – Abs_{sample}) / Abs_{blank} x 100

III. RESULTS

Amongst two varieties of dates, the highest percent yield 15.676% was obtained by methanol for *Zahedi* pits and 15.460% for *Ajwa* pits, followed by acetone 13.248%, aqueous 10.003%, butanol 9.867%, hexane 9.151%, chloroform 8.905% for *Ajwa* pits and aqueous 10.396%, acetone 8.339%, Chloroform 2.802%, and hexane 1.487% for *Zahedi* pits.

Figure 1: The total extract yield expressed in percentage %



TPC and TFC of two varieties of Phoenix Dactylifera L. of different extracts of pits of *Phoenix dactylifera* L. varieties; *Ajwa* and *Zahedi* were determine and results are shown in Fig. 2 and 3.

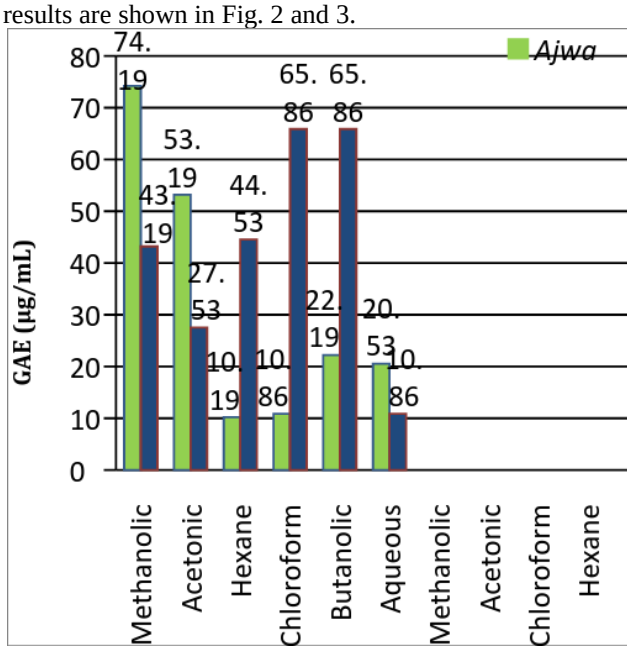


Figure 2: Comparison of GAE for all extracts of *Ajwa* and *Zahedi* varieties to evaluate TPC

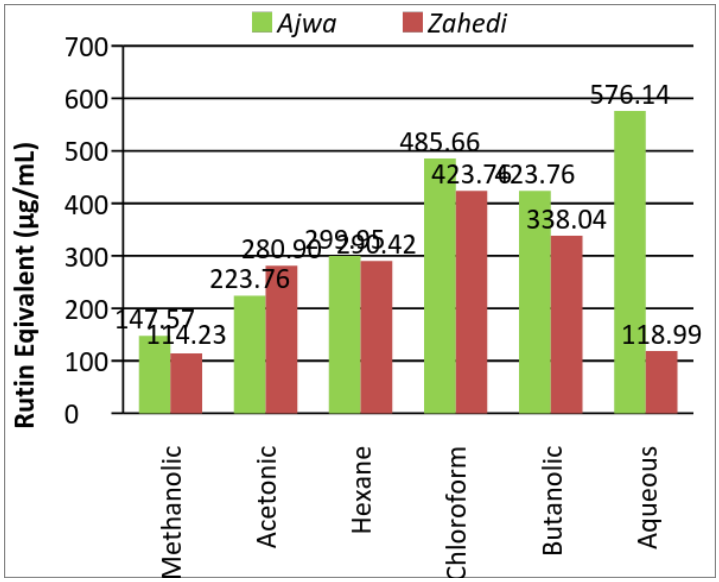


Figure 3: RE in µg/mL (or mg/g dried sample) for all extracts of pits of *Ajwa* and *Zahedi* dates, to estimate TFC.

The capacity of pits of *Ajwa* and *Zahedi* dates of *Phoenix dactylifera* L. to scavenge DPPH radical was evaluated and the results are shown in Table 1. The concentration of each species to scavenge 50% DPPH radical (EC_{50}) was also calculated for the evaluation of the effectiveness of the given samples as an antioxidant and was compared with the result of Standard (Gallic Acid).

Table 1: DPPH inhibition % at concentration of 1mg/mL and EC50 of extracts of Ajwa and Zahedi pits

DPPH scavenging (%)			EC50 (mg/mL)	
Extracts	**Ajwa**	**Zahedi**	**Ajwa**	**Zahedi**
Methanolic	**80.93**	**82.82**	**0.52**	**0.68**
Acetonic	**85.48**	**83.32**	**0.41**	**0.45**
Hexane	**18.07**	**9.76**	**4.24**	**8.32**
Chloroform	**0.11**	**5.44**	**-**	**-**
Butanolic	**22.17**	**79.28**	**2.5**	**0.357**
Aqueous	**72.11**	**42.07**	**0.599**	**0.7**
Gallic Acid	**97.86**		**0.097**	

The free radical scavenging action as per DPPH of all extracts of *Ajwa* and *Zahedi* pits with correspondence in standard (Gallic Acid) at concentration of 1mg/mL, is also shown in Table 1.

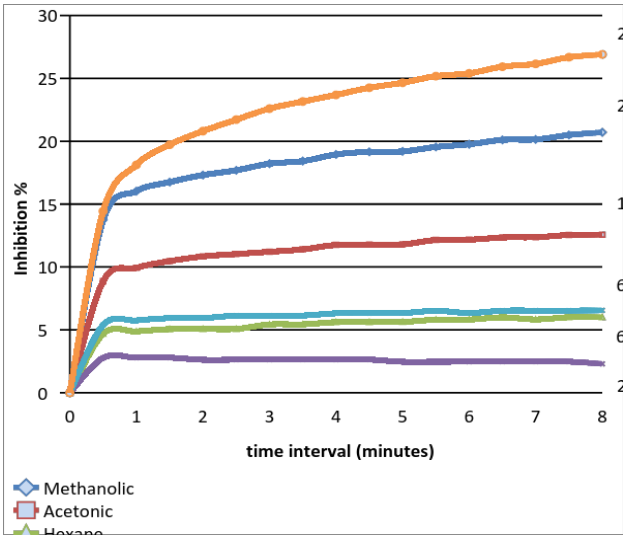


Figure 4a: Percentage inhibition of ABTS^{+} radical by different extracts of *Ajwa* pits extracts over 8 minutes time period. Absorbance was recorded after 30 seconds at 730 nm.

ABTS^{+} radical scavenging was estimated by calculating percentage inhibition over 8 minutes as well as 24 hours time period and are shown in Fig.4(a, b) and Table 2 respectively. TEAC values of various extracts of *Ajwa* and *Zahedi* pits, ranges from 64.26-67.35 µM, shown in Fig.5. TEAC values also verified prominent antioxidant capacity of aqueous extracts of *Ajwa* pits whereas acetonic extracts of *Zahedi* pits showed greater values among all of its remaining extracts.

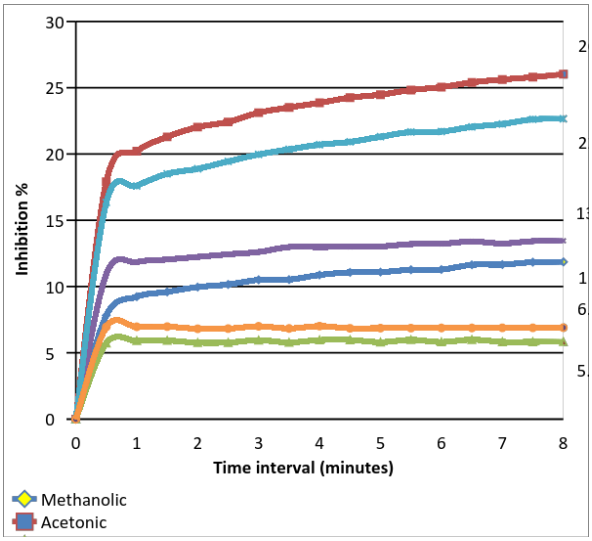


Figure 4b: Percentage inhibition of ABTS^{+} radical by different extracts of *Zahedi* pits over 8 minutes time period. Absorbance was recorded after 30 seconds at 730 nm.

Table 2: Percentage inhibition of ABTS^{+} radical as a function of time for different extracts of Ajwa and Zahedi dates pits with the duration of 24 hours.

Sample	Extracts	Percentage Inhibition of ABTS radical after			
		0 hour	24 hours	48 hours	72 hours
Ajwa	Methanolic	20.71	80.18	23.99	6.25
	Acetonic	12.57	79.72	94.81	28.13
	Hexane	6.02	59.91	7.46	40.63
	Chloroform	2.30	15.67	25.12	56.25
	Butanolic	6.55	7.37	91.90	46.88
	Aqueous	26.90	2.76	95.62	31.25
Zahedi	Methanolic	11.86	8.76	5.19	18.75
	Acetonic	26.02	5.99	87.52	53.13
	Hexane	5.84	6.45	75.69	59.38
	Chloroform	13.45	9.68	73.91	59.38
	Butanolic	22.65	70.05	25.45	31.25
	Aqueous	6.90	4.61	90.76	59.38

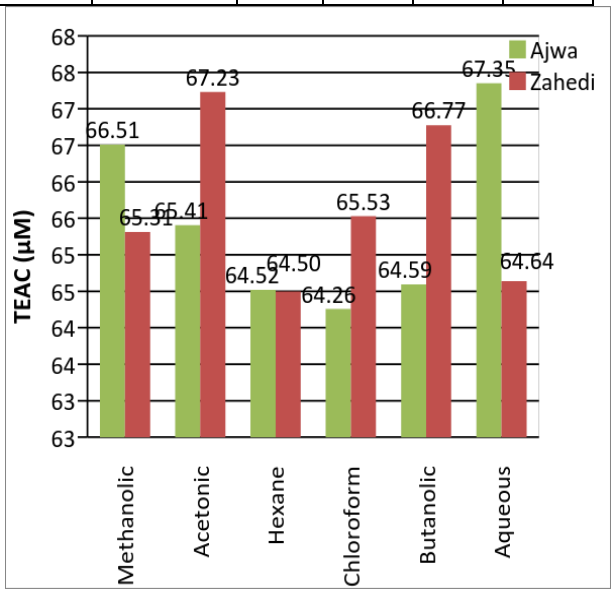


Figure 5: Trolox equivalent antioxidant capacity (TEAC) of all extracts of *Ajwa* and *Zahedi* pits, expressed in µM.

The results of relative analysis between reducing power capacity (RPC) of *Ajwa* extracts and *Zahedi*

extracts of all with respect to standard (Gallic acid) are shown in Fig. 6.

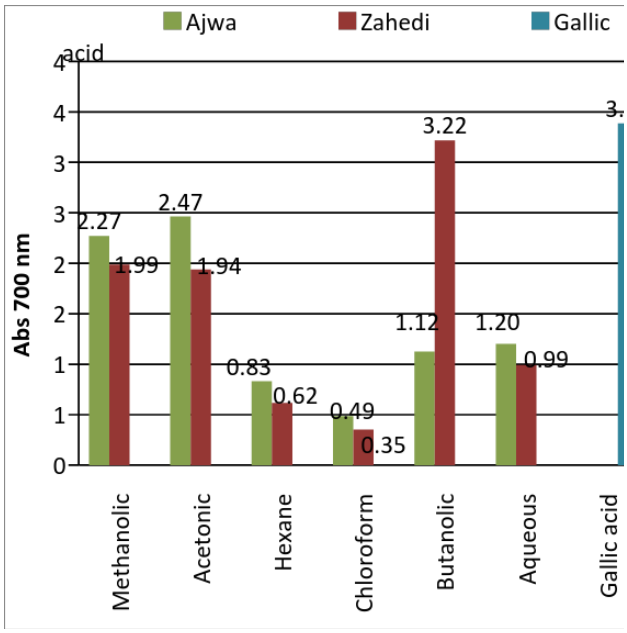


Figure 6: Comparison of RPC of all extracts of *Ajwa* and *Zahedi* pits.

The free radical scavenging potential of methanolic, acetonic, hexane, chloroform, butanolic and aqueous extracts of *Ajwa* and *Zahedi* pits, using phosphomolybdate assay are shown in Fig. 7

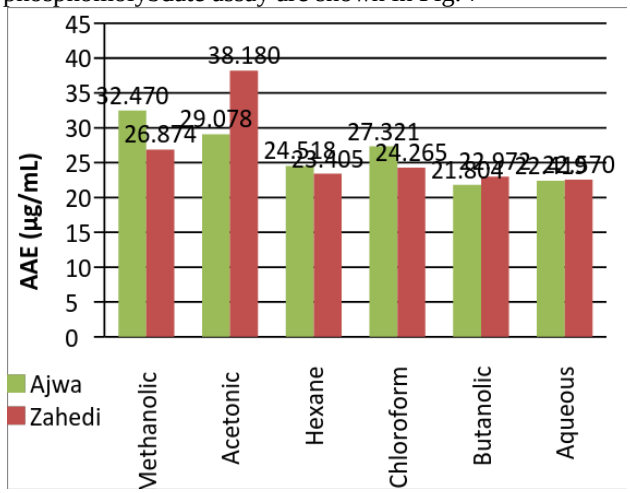
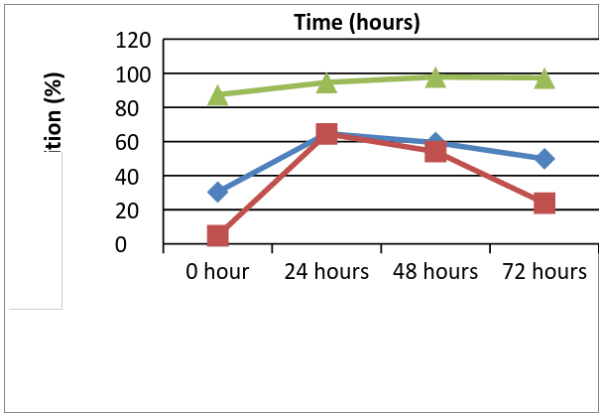
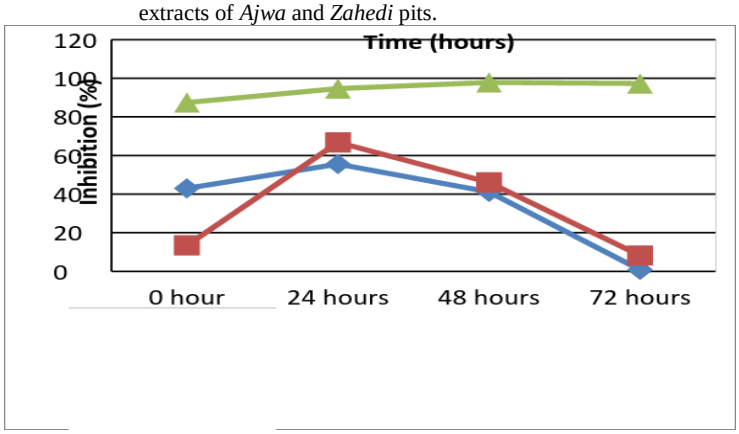


Figure 7: Comparative determination of ascorbic acid equivalent for all extracts of two *Phoenix dactylifera* L. varieties; *Ajwa* and *Zahedi*.

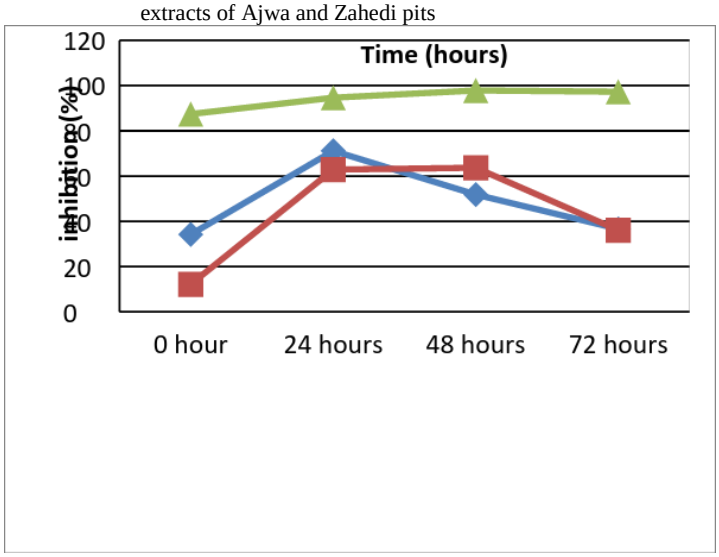
Antioxidant protective outcome against lipid peroxidation was determined by ferric thiocyanate method. Absorbance was recorded after 24 hour interval for four days and results are compared with standard (BHA), shown in 8(a-f)



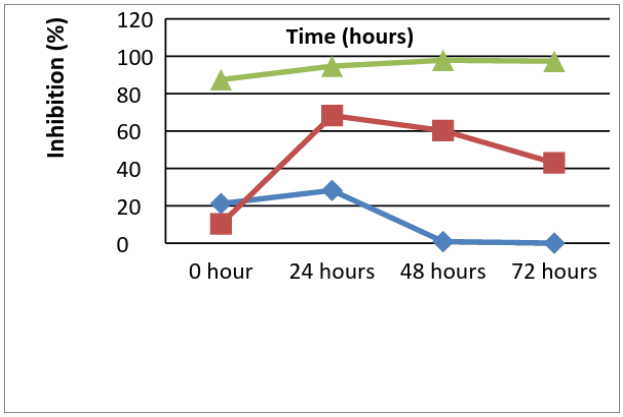
Figures 8(a) Lipid peroxidation inhibition by methanolic extracts of *Ajwa* and *Zahedi* pits.



Figures 8(b) Lipid peroxidation inhibition by n-hexane extracts of Ajwa and Zahedi pits

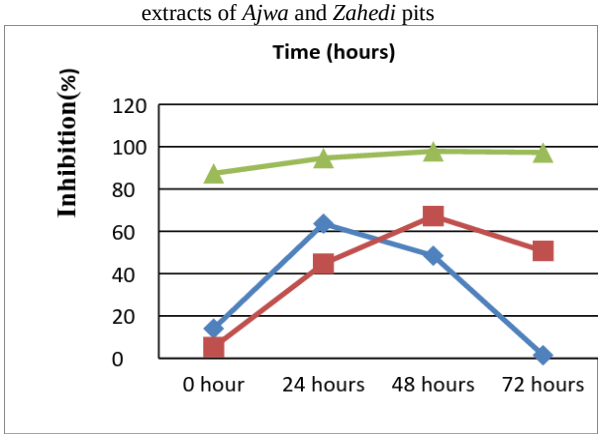


Figures 8(c) Lipid peroxidation inhibition by acetonic extracts of *Ajwa* and *Zahedi* pits

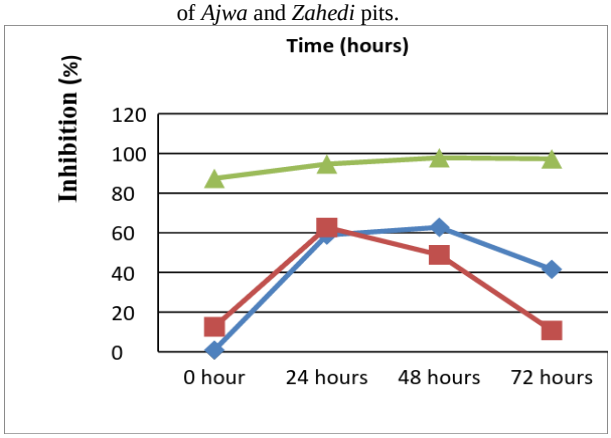


Figures 8(d) Lipid peroxidation inhibition by chloroform extracts of *Ajwa* and *Zahedi* pits

Figures 8(e) Lipid peroxidation inhibition by butanolic extracts of *Ajwa* and *Zahedi* pits



Figures 8(f) Lipid peroxidation inhibition by aqueous extracts of *Ajwa* and *Zahedi* pits.



IV. Discussion and Conclusion

Discussion:

From the immemorial time, fruit had been taken as nutritive and healthful diet. Fruits and vegetables are always computed as a crucial need of human diet. Seasonal fruits ingestion is always advised. High intake of fruits and vegetables decreases the risk of degenerative diseases [15, 16]. Fruits are considered as a good source of antioxidants at varying efficacy. All dates varieties named with a botanical nomenclature; *Phoenix dactylifera* L. because of its extreme diversity. Madinah Munawarah is one of the city of kingdom of Saudi Arabia produced some varieties of dates like *Amberah* and *Ajwa.* Ajwa date is a special luxury type of dates with black color, soft and delightfully fine taste, which is special because of its nutritional as well as religious significance [17]. It is only cultivated at Madinah Munawarah, Saudi Arabia. There are a very few reports on the antioxidant activities of *Ajwa*. The current comparative study is the first ever study planned to explore an inclusive antioxidant capacity profile of fruit and pit of *Ajwa* and compare it with *Zahedi* of *Phoenix dactylifera* L.

A number of methods and modifications have been proposed to determine antioxidant potential.

The TPC of the extracts were measured by Folin-Ciocalteu's reagent which is a mixture of phosphomolybdate and phosphotungstate. The reduction of tungsten and molybdenum oxides gives blue color to solution, which can be examined spectrometrically at 765 nm. The results were determined in Gallic acid equivalent by reference to standard curve. ($y = 0.001x+0.1411$, $r^2 = 0.996$). In the current analysis, methanolic extract of *Ajwa* pits yield highest GAE i.e., 74.19 ± 0.026 µg/mL which is followed by acetonic (53.19 ± 0.025), butanolic (22.19 ± 0.01), aqueous (20.53 ±0.006), chloroform (10.86 ± 0.028) and hexane (10.9 ± 0.002) extracts as shown in table 1. On the other hand, in case of *Zahedi* pits, GAE was observed to be greater in chloroform and butanolic extracts (65.86 ± 0.01 µg/mL) which is followed by hexane (44.53 ± 0.007) > methanolic (43.19 ± 0.006) > acetonic (27.53 ± 0.005) > aqueous (10.86 ± 0.007 µg/mL) extracts. Total phenolic content of *Zahedi* pits reported by Ardekani *et al.*, 2010 was found to be close in aqueous extract (11.61 ± 1.04 mg/g of dried sample) but low in case of methanolic extracts i.e., 14.31 ± 0.95 mg/g of dried sample. Polyphenols are responsible for high antioxidant activity [18, 19, 20].

Flavonoids are polyphenolic compound which has scavenging ability like other antioxidants present in the plants. Hydroxyl group present at C-3 and C-5 or central ring stabilize the radicals. Flavonoids such as flavonols and flavonols reduce risk of cardiovascular diseases and other oxidative stress [21]. Plants having high flavonoid content may be considered to a suppressor for cardiovascular diseases. In the current studies total flavonoid content of all the extracts of *Ajwa* and *Zahedi* pits were evaluated in accordance with an already reported procedure [22], and the results were repoted as Rutin equivalent by refrence to standard curve (y = 7E-5x + 0.0531,

r^2 = 0.9908). The aqueous extract of *Ajwa* showed highest RE value (576.14 ± 0.006) as compared to other extracts. Among the extracts of *Zahedi*, chloroform extracts showed highest flavonoid content i.e., 423.76 ± 0.003 µg/mL of RE. Overall, *Ajwa* exhibited the highest values of TFC.

The potential of flavonoids to act as antioxidant depends upon their molecular structure. The position of OH group and other features in chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities [23]. We can say to some extent that our study is in covenant with Sengul et al.,[24] which reported no relationship between TPC and AOC. It cannot be ignored that AOC was not exclusively from Phenolic contents but also possible due to the presence of some other phytochemicals which may contribute to the AOC. This needs further extensive investigations because Folin-Ciocalteu method is not an absolute measurement of total phenolic materials. There is different range of phenolic compounds with different antioxidant potentials which are structure dependent.

The phosphomolybdate assay evaluates the capacity of a sample to obliterate an unimpeded radical by transferring an electron to the latter. Ascorbic acid equivalent is recorded by mentioning standard curve (y = 0.0025x -0.0319, r^2 = 0.9955). The key principle of the phosphomolybdate assay is focused on the reduction of molybdate (VI) to molybdate (V) by the influence of bio-reducing agent in the sample. This assay is pragmatic to evaluate the reducing capacity of the raw date extracts as well as the pure compounds. Presence of antioxidant reduces Mo (VI) into Mo (V) which gives green color on high temperature in an acidic condition. The complex formed has maximum absorbance at 700 nm. Intensity of color indicates the amount of reduction by the antioxidants. It is estimated that acetonic extract of *Zahedi* pit has highest equivalent (38.18 ± 0.05 mg/mL) of ascorbic acid. Overall, extracts possess reducing ability. It ranges from 38.18 (acetonic extracts of *Zahedi* pits) to 21.804 (butanolic extract of *Ajwa* pits).

A number of methods are used to evaluate the radical scavenging effects of antioxidants. DPPH: 2, 2-diphenyl-1-picrylhydrazyl is a dark-colored crystalline powdered composed of strong and stable free radical molecules. DPPH promptly is rummaged by antioxidants under normal conditions. It is known that antioxidants in food are water soluble, fat soluble, insoluble, or bound to cell walls and thereby not inevitably easily accessible to react with DPPH. Consequently they react at different rates i.e. differing kinetics, and the reaction will frequently not go to consummation in an equitable assay time. Therefore, the sample size that can lower the preliminary absorbance of DPPH solution by 50% has been preferred as the endpoint for computing the antioxidant potential. This change is equated to the change induced by Gallic Acid, the reference standard, and the antioxidant activity of the sample is calculated. Effectiveness of antioxidants was estimated by degree of decolorization. Various extracts of *Ajwa* and *Zahedi* pits were evaluated to check their scavenging potential against DPPH radical with a dose of 1mg/mL concentration. The scavenging potential was expressed in inhibition percentage. Results showed that acetonic extracts of *Ajwa* pits had significantly higher inhibition (85.48%) among all extracts of both date seeds. However, chloroform extract of *Ajwa* shows least inhibition (0.11%). Inhibition for overall extracts ranges from 85.48 % to 0.11 %. Hexane and chloroform extracts have least anti radical activity representing low content of antioxidants. Efficient concentration (EC_{50}) corresponds to amount at which antioxidants present inside the extracts possess 50 % inhibition. Lower the values of extracts demonstrate effectiveness at lower concentration. It was observed that butanolic extracts of *Zahedi* pits was more efficient than other extracts. Order of EC_{50} is as followed. Acetonic > methanolic > Aqueous > butanolic > hexane (*Ajwa* pits),

Butanolic > acetonic > methanolic > aqueous > hexane (*Zahedi* pits).

The ability to scavenge ABTS radical is directly proportional to the antioxidant capacity. 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot -}$) is engendered by oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants. $ABTS^{\cdot +}$ is a decolorization assay in which $ABTS^{\cdot +}$ radical undergoes reduction course and the bluish green color of the radical is scavenged comparable to the amount of antioxidant values in the extract. The impacts of both the concentration of antioxidant and extent of reaction on the inhibition of the radical cation absorption are taken into justification when determining the antioxidant activity. Aqueous extracts of *Ajwa* had highest inhibitory effect (26.90%). On the other hand, acetonic extract of *Zahedi* pits had significant inhibition (26.02 %). Order of inhibition, by the extracts over 8 minutes with the interval of 30 seconds, was as: Aqueous > methanolic > acetonic > butanolic > hexane > chloroform (*Ajwa* pits),

Acetonic > butanolic >chloroform > methanolic > aqueous >hexane (*Zahedi* Pits).

Inhibition was observed to be highest after 24 hours interval; antioxidants present inside extracts scavenged to maximum after 24 or 72 hours and then decreased in inhibition were observed. Overall, both

varieties exhibited effective $ABTS^{+}$ scavenging activity thus betokening their potency against reactive oxygen species-led damages.

Lipid peroxidation, which is widely accepted as primary toxicological test, is triggered by the generation of free radicals from a variety of sources including organic hydro peroxides, redox cycling compounds and iron-containing compounds. Lipid profile was sourced by linoleic acid emulsion. Antioxidants inhibit the formation of peroxyl radials and hence, estimated by remaining ferric-thiocyanate complex formed by radicals. [25] Inhibition pattern followed by standard (BHA) had shown gradual rising but in case of date seed extracts, scavenging percentage increased with increase in incubation time, reached to point at which antioxidants exhibited paramount inhibition then, decrease rapidly. Startling behavior might be due to presence of some intrusive compounds like sugars [26]. Effective inhibition had been shown by acetonic extracts of *Ajwa* pits after 24 hour (71.14 %). Then decrease to 51.825 % and hence, 36.63% after 72 hours interval. In case of *Zahedi* extracts, highest inhibition had been exposed by chloroform extracts (68.24%) which is not found as a good antioxidant previously. In the reducing power assay, the presence of antioxidants in the different extracts of *Ajwa* pits and *Zahedi* pits were able to transform the oxidized form of Fe^{3+} into Fe^{2+} which was assessed by the intensity of the resultant Prussian blue color complex. With the increase of concentration, the absorbance of the extracts and the standard were found to be increased gradually [27].The reducing capacity is generally associated with the presence of reductones and the antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom [28]. The result indicated that the marked reducing power of different extracts of *Ajwa* pits and *Zahedi* pits seems to be due to the presence of polyphenols which may act in a similar fashion as reductones. It was also observed that this assay is sensitive to pH; change in pH lead to either complete inhibition with light pink color indication or intense red color with low inhibitory effect. It was perceived that the butanolic extract of *Zahedi* pits observed to be close (3.22 ± 0.10) to standard gallic acid (3.39 ± 0.01). Chloroform extract of Zahedi pits had least absorbance (0.35 ± 0.01), overall it exhibit least anti-radical as well as antioxidant potential. This may be due to least polar nature, incompatible to antioxidants like phenolics.

Conclusion:

In this current study we mainly focused on comparative evaluation for antioxidant potential of pits of *Ajwa*; an emerging plant in pharmacological research and *Zahedi* varieties of *Phoenix dactylifera* L. Both varieties have significant antioxidant activity. This potential was analogized with their phenolic and flavonoid contents. Precisely, the methanolic extract of pits of *Ajwa* and chloroform extract of *Zahedi* are more potent antioxidative and radical scavenging due to having high TPC and TFC as compared to other extracts. Scavenging of DPPH and ABTS radicals and other antioxidant assays also showed highest effectiveness of the methanol and acetonic extracts. Date stones are usually considered as a waste material but possess antioxidant and anti-aging properties and hence, are useful [29, 30]. Epidemiological manifest proposes an inverse relationship between dietary intake of flavonoids and cardiovascular risk. An accumulated evidence of experimental study indicates high flavonoid content in pits of *Ajwa*, which might lower the risk of cardiovascular diseases in people. The consumption of dates has positive effects on human health and the results of these findings suggests that it can also leads to produce a useful commercial drug after identification and isolation of active components that will assist in the treatment of cardiac, gastric and neuronal diseases [31]. Further studies are mandatory which will primarily focus on detection, isolation, determination and characterization of active components, effective in cardiac problems. This link would open a new era of research on drug discovery and development on heart diseases cured by *Ajwa* date.

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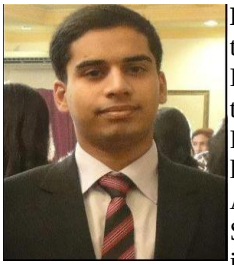

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