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Determination of Antioxidant Capacity, Total Phenolic and Flavonoid Contents of Zahdi Date Seed and Their Effect on Beef Patties

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Abstract. The study included preparation of aqueous (As) and alcoholic (ethanolic Es , methanolic Ms) and oil extracts (Os) of Zahdi date seeds (Zs)(Phoenix dactylifera) . Chemical content of date seed was studied and the total content of phenols and flavonoids were estimated for prepared extract. Bioactive compounds of this extract was identification by Gas Chromatography Mass Spectrometer GCl MS. Antioxidant activity , reducing power, chelating of iron ions of extract was studied , then used the extract which gave highest antioxidant activity in beef patties with two concentrations 0.05% and 0.1% to test their efficiency in oxidative inhibition and prolonging the reservoir age of stored beef patties in refrigerated for 10 – days and following chemical indicators in Peroxide value(PV) , Thiobarbituric acid(TBA) in treated beef patties . Moisture , protein ,oil, ash and carbohydrate contents of Zs 9.36% , 4.87% , 8.56% , 1.34%, 75.87% respectively . All extracts containing many bioactive compounds that identification of GC-MS and differented in their percentage according to the type of extract. All extracts share with some bioactive compound as 1-(+)-Ascorbic acid 2,6-dihexadecanoate, gamma.sitosterol ,Octadecanoic 5-Methyl-2-ethylamino-2-thiazoline, acid , Hexadecanoic acid and 2-hydroxy-1-(hydrixymethyl)ethylester . Alcoholic extracts (Es and Ms) exhibited the highest content of phenolic compound compared with As was 67.32mglml for Es , Ms was 65.32mglml While the total content of flavonoids for As 33.32mglml and Es 52.16 mglml , 46.16mglml for Ms , As and alcoholic extracts of Zs exhibited antioxidant activity was 88.70% for Es and 87.02% for Os , 85.22% for Ms , but As was the lowest 43.01% . The prepared extracts showed the higher reducing power was 2.63 and Chelating of iron ions 60.93 % and for Es , There was a significant decrease ($P<0.05$)in peroxide value and thiobarbituric acid in beef patties treated with ethanolic extracts compared with control.

Keywords. Total phenolic content, Zahdi date seed, Antioxidant activity , Lipid oxidation.

I. INTRODUCTION

New studies found that Date seeds contain valuable bioactive compounds such as Phenolic Compounds , Flavonoids , Antioxidants and Fibre dietary , Percentage these compounds was highest than that presenting in flesh part , and that high levels of α-Tocopherol , Ascorbic acid , Glutathione , and Sinapic acid , Caffeic acid, Protocatechenic acid as Polyphenol compounds [1-2] , and identificated many of aromatic compounds in date seed such as alcohols ,esters ,aldehydes, terpenes ,ketones , saturated and unsaturated hydrocarbons [3] . Oxidation due to free radical that considers is the main factor of disease , Scientific evidence show that oxidative stress is a cause of fat oxidation [4] , functional change of endothelial that leading to atherosclerosis , All combinations of active compounds found in date seed are assumed to be able to affect lipid profile by increasing HDL level and decreasing total cholesterol , triglyceride and LDL level , thus atherogenic index will decrease as well [5] . Date seeds that has been considered as a good example of functional food and their a large role of food avidities and extend of shelf life [6] .This study came with the aim of knowing the effect of date seed extracts as a natural antioxidant to be used in date seed extracts as food additives to delay the oxidation of fats in various foods, especially meat products.

II. MATERIALS AND METHODS

- *Plant Material*

Date seed obtained from date fruit (*Phoenix dactylifera* var. Zahdi) were procured from local market, Basrah. Dry seeds were ground into a fine powder using a blender (binder USA) and sieved through stainless steel sieve of 40 mesh . The powder was placed in polyethylene bags and stored in a refrigerator until use , and were procured beef from Basrah .

FIGURE 1. Photographs of date and seed.

- *Chemical Composition of Date Seed*

Moisture , protein , oil ,Ash and carbohydrate contents of date seed were determinate according to the Association of Official Analytical Chemists [7] .

- *Preparation of Date Seeds Extract*

A- Water Extracts

Followed of method [8] , Date seed powder (20g) was mixed with500ml of distilled water . Thereafter ,the mixtures were placed in shaking incubator for 24 hours at 28C˚ then centrifuged at 2500 rpm for ten minutes, then filtered using funnel with Whitman No.1, 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, Put in glass containers and refrigerated at 4C˚.

B- Ethanolic Extract

Preparation of ethanolic extracts by weighted 100g of date seed powder which dissolved by 500ml ethanol (70%) and mixed them well and left for 24 hours at laboratory temperature (25-30C˚). The mixtures were filtrated by whatman No.1 and concentrated by rotary vacuum evaporator at 40C˚and left at laboratory temperature to get dry powder . Put in glass containers and refrigerated at 4C˚[9] .

C- Methanolic Extract

Followed steps in paragraph B to get at ethanolic date seed extracts.

D- Oil Extract

Date seed oil was extracted from date seed powder by the method of [10] , A weighed 10g of Sample and was solved in 20 ml of chloroform , 40ml methanol and 1ml distilled water and mixed them well by glass rood for five minutes. Then , 20 ml of chloroform was added to mixture and mixed well for other five minutes . Thereafter , added to 20ml distilled water with mixing and left for 15 minutes , Then was filtered by whatman No.1 , The mixture placed in separation funnel , Afew minutes later , two phase was formed . The lower chloroformic phase containing the total lipids was dried and calculated by weighing a dry beaker and beaker with oil after chloroform evaporation .

- *Determination of Total Phenols*

Total phenols in zahdi date seed was determined calorimetrically at 760 nm with the Folin- Ciocalteau reagent as previously done by [11] .

- *Standard Curve*

Standard solution from Gallic acid used for calculated Total phenols in extract at concentration ranged from 10 to 100mg/ml dependent on graphic relationship between Acid concentration and Absorption at 760nm wave length (Figure 2)

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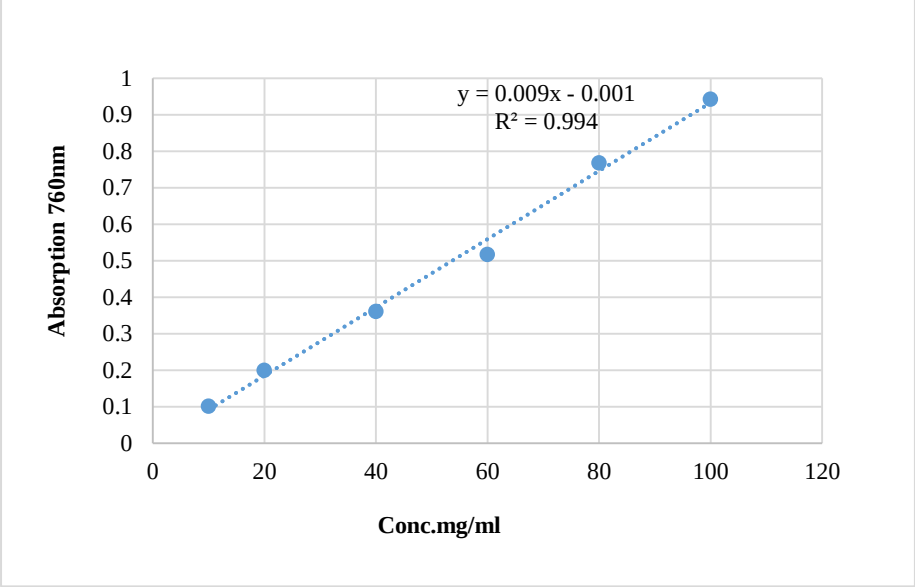

FIGURE 2. Standard curve of Gallic acid.

- *Flavonoids Determination of Total*

Date seed powder (1g) was dissolved in 1.5ml ethanol , added to it an equal volume of Aluminium chloride $AlCl_3.6H_2O$ (2%) which prepared in 100ml methanol, mixed the ingredients well and the absorbance was determined at 415nm.

- *Standard Curve*

A standard curve was prepared using rutin in the range (10-200mg/ml) . Thereafter , Flavonoids content was determined through the graphic relationship between rutin concentration and absorption at 415nm (Figure3).

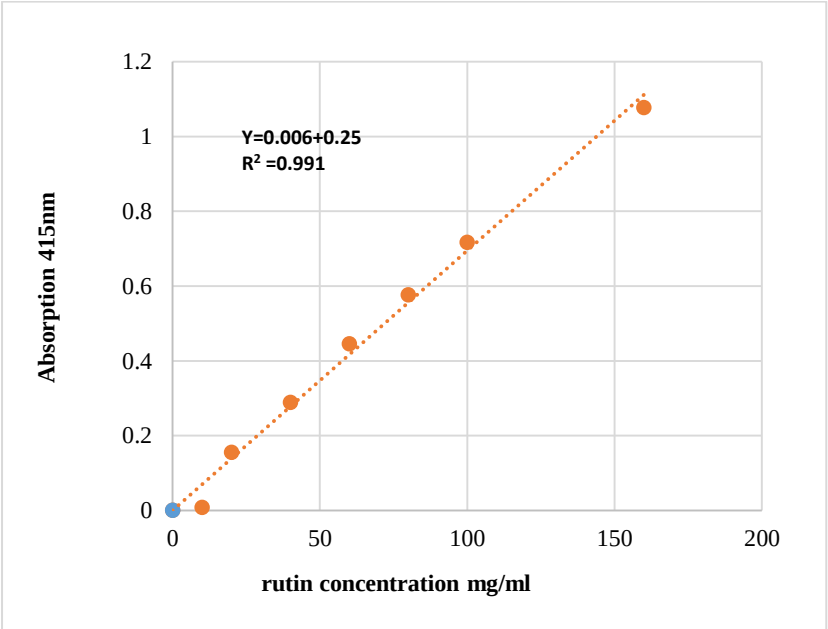

FIGURE 3. Standard curve of rutin.

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- *GC/MS Analysis*

Bioactive compounds of the zahdi seed extracts were determined by GC/MS analysis in GC/MS Laboratory /Agriculture college / Basrah university . A Gas Chromatograph QP210 ULTRA (Make : SHIMADZU , Japan). The GC/MS instrument conditions are given in Table 1.

TABLE 1. Operating conditions for the GC/MS.

Mass Spectrometer	Gas Chromatography
Ionsource Temp : 200.00˚c Interface Temp : 250.00˚c Solvent Cut Time : 3.00 min Start Time : 3.00 min End Time : 28.00 min ACQ Mode: Scan Event Time : 0.50 sec Scan Speed: 1000 Start m *l* z : 50.00 End m *l* z : 500.00	Column Oven Temp :40˚c Injection Temp : 250.00˚c Injection Mode : Split Flow Control Mode: Linear Velocity Pressure : 49.5kpa Total Flow : 34.0ml*l* min Column Flow : 1.00 ml*l*min Linear Velocity : 36.1cm *l* sec Purge Flow : 3.0 ml *l* min Split Ratio : 30.0

- *Measurement of Antioxidant Activity*

A- DPPH (1,1-Diphenyl-2-Picrylhydrazyl) Free Radical scavenging Activity Assay:

The free radical scavenging activity of the extract , based on scavenging of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated according to the procedure described by [12] . A sample of the extract (1ml) was mixed with 4ml methanol and 1ml of reagent solution (10mM of DPPH in ethanol) . The control contained only DPPH solution in place of the sample. The mixture was vigorously shaken and left to stand at room temperature . After 30 minutes the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517nm . The scavenging effect was calculated using the expression **:**

$$\text{Inhibition\%} = \{1 - [\text{Abs sample} / \text{Abs control}]\} \times 100$$

B- Reducing Power Assay :

0.25ml sample of Zs extract was mixed with phosphate buffer (0.25ml) and potassium ferricyanide (2.5ml). The mixture was incubated at 50º C for 20 min. Aliquots of trichloroacetic acid (2.5ml) were added to the mixture , which was then centrifuged at 3000rpm for 10 min . The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and a freshly prepared ferric chloride solution (0.5ml) . The absorbance was measured at 700nm . A control was prepared without adding extract . Increase in absorbance of mixture was indicated high reducing power [13].

C- Chelating Activity of Ferrous Ion :

Chelating activity towards Fe^{+2} was measured by the method of [14]. 1ml of Zs extract was mixed with ethyl acetate (3.7ml) . 0.1ml of ferrous chloride (2mM) was added to mixture , and 0.2 ml of 8- Hydroxy quinoline(5mM) , The mixture was mixed and left at room temperature for 10min. The Absorbance was measured at 562 nm , EDTA2Na was used as standard . A control was prepared without adding extract . Chelating activity was calculated using the expression :

$$\text{Chelating activity\%} = \{1 - [\text{Abs sample} / \text{Abs control}]\} \times 100$$

- *Using of Ethanolic Extract as Antioxidant in Beef Patties*

A- Preparation of Beef Patties

The Beef were purchased from the local market in Basrah , Beef patties were prepared after wished it and minced using a meat grinder . Then, added it 10% of fat and 2% salt . Meat mince was devided into 3 sample : 1-First sample : it was control without adding extract , 2-Second sample : ethanolic extract was added (0.05%) , 3- Third sample : ethanolic extract was added (0.1%) . Beef patties were placed in polyethylene bags and stored in refrigerator at 4ºC for 10 days . At designated time (0,3,7, and 10 days) , samples were taken for determination of peroxide value(PV) [15] and thiobarbituric acid –reactive substances (TBARS) [16] .

- *Statistical Analysis*

All experiments were carried out in triplicate . Data were subjected to the analysis of variance (ANOVA) . Statistical analysis was carried out using the Statistical Package analysis for Social Science (SPSS) [17] .

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III. RESULTS AND DISCUSSION

- *Chemical Composition of Zahdi Date Seed*

Table 2. presents the average chemical composition of Zahdi date seed . Zs contained 9.36% moisture . Concerning the crude lipid contents , this value was 8.56% and it is comparable to the results reported by [18] at studied chemical composition of Zahdi date seed were found %8.14 , Protein were 4.87% , our results are smiliar to those from [19] , who reported a protein content 4.71% in Khalas date seed . The ash content was 1.34% and a comparable result was found by [20] (1.20%) and [21] (1.25%) . The total carbohydrates content was found 75.87% .

TABLE 2. Proximate composition of Zahdi date seed.

Component %	Composition of date seed
Moisture	9.36
Lipid	8.56
Protein	4.87
Ash	1.34
Carbohydrate	75.87

- *GC/MS Analysis*

A - Aqueous Extract Composition of Zahdi Date Seed

The identification of Aqueous extract composition(As) of Zs was performed by Gas Chromatography Mass Spectrometry (GC/MS). The result is shown in figure4.

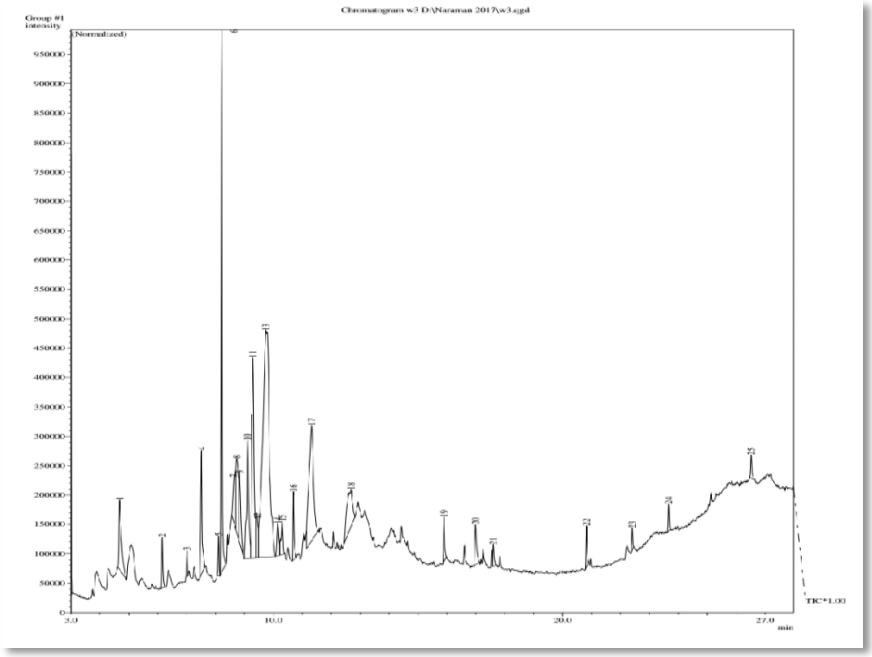

FIGURE 4. GC/MS diagram of As.

The Aqueous extract composition of the Zs that detected through the study are listed in the table . The results show that the 3-methyl mannoside had the highest amount 30.54% while 4-Ketopimelic had the lowest amount 0.39 % , Also , the results show another bioactive components like 4H-pyran-4-one,2,3-dihydro-3,5dihydroxy-6-methy and gamma-sitosterol that the percentage are 11.70% and 0.57% respectively .

TABLE 3. Zahdi date seed composition.

No	Identified compounds	Retention time (min)	Weight %
1	Ethanamine, 2-methoxy-N-(2-methoxyethyl)-N-methyl-	4.680	3.39
2	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	6.155	1.04
3	4-Ketopimelic	7.010	0.39
4	Cyclohexanamine, N-3-butenyl-N-methyl-	7.514	3.46
5	Ethanamine, N-ethyl-N-nitroso-	8.105	0.77
6	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8.210	11.70

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No	Identified compounds	Retention time (min)	Weight %
7	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	8.633	2.65
8	2(3H)-Furanone, 5-ethyldihydro-	8.732	3.50
9	1,2-Benzenediol	8.825	2.44
10	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	9.105	5.43
11	2-(1-Hydroxyethyl)-2-methyl-1,3-oxathiolane	9.289	8.06
12	1-Propanol, 3-chloro-, acetate	9.450	1.94
13	3-Methylmannoside	9.742	30.54
14	Butanedioic acid, monomethyl ester	10.152	1.26
15	Butanedioic acid, 2-hydroxy-2-methyl-, (S)-	10.303	1.33
16	'-(3H-Indol-3-ylmethylene)-hydrazino]-5-methyl-[1,2,4]triazol-4-ylamine	10.690	1.52
17	Sucrose	11.319	10.77
18	Ethyl trans-3-methyl-2-oxiranecarboxylate	12.686	4.60
19	Pentadecanoic acid	15.897	0.98
20	Pent-1-en-3-one, 4,4-dimethyl-1-(4-morpholino)-	16.990	1.43
21	6-Octadecenoic acid, (Z)-	17.605	0.50
22	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	20.826	0.79
23	Octadecanoic acid, 2,3-dihydroxypropyl ester	22.412	0.44
24	Hexatriacontane	23.667	0.52
25	.gamma.-Sitosterol	26.519	0.57

B- Ethanol Extract Composition of Zahdi Date Deed

The ethanolic extract composition of the Zs that detected through the study are listed in the table 4.and Figure5. The results show that the 1-(+)-Ascorbic acid 2,6-dihexadecanoate had the highest amount 18.09% and Hexatriacontane 14.12%, Also , the results show another bioactive components like Quinoline,2-methyl,1-oxide and gamma-sitosterol are 2.09% , 3.04% respectively . Our results was in agreement with those found by [22], who identified essential oil composition was extracted from Capers (*Capparis spinosa*) by GC/MS As formation Hexatriacontane are 15.87 % , This compound are possess many of biological properties such as antioxidant , antibacterial and therapeutic agent .

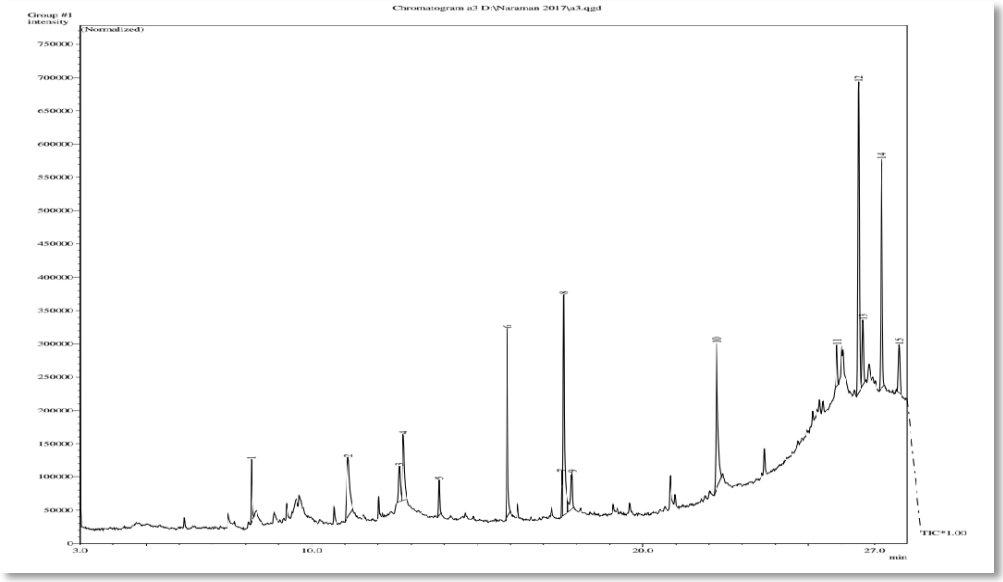

FIGURE 5. GC/MS diagram of Es.

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TABLE 4. Es .Zahdi date seed composition.

No	Identified compounds	Retention time (min)	Weight %
1	Ethanamine,2-methoxy-N- (2-methoxyethyl)-N-methy	8.178	2.73
2	4-H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methy	11.096	7.51
3	2-Furancarboxaaldehyde,5-(hydroxymethyl)-	12.649	2.57
4	2-(1-Hydroxyethyl)-2-methyl-1,3-oxathiolane	12.761	5.26
5	5-Methyl-2-ethylamino-2-thiazoline	13.848	1.57
6	Ethylene glycol butyl ether , trimethyl silyl ether	15.901	8.48
7	Quinoline, 2-methyl- 1-oxide	17.550	2.09
8	2-Butanone, 4-hydroxy-3-methyl	17.613	13.20
9	1,2-Benzenediol	17.866	3.61
10	Ethyl trans-3-methyl-2-oxiranecarboxylate	22.230	11.91
11	Sucrose	25.863	2.18
12	1-(+)-Ascorbic acid 2,6-dihexadecanoate	26.522	18.09
13	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethy ester	26.652	3.63
14	Hexatriacontane	27.212	14.12
15	gamma. sitosterol	27.742	3.04

C- Methanol Extract Composition of Zahdi Date Seed

The methanolic extract composition of the Zs that detected through the study are listed in the table 5.and Figure 6. The results show that the 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl had the highest amount 39.18% follwed Hexadecanoic acid ,2-hydroxy-1-(hydroxymethyl)ethylester 27.48% and Octadecanoic acid,2,3-dihydroxypropylester was called 1-Monostearin or Glycerol monostearate (GMS) that using as emulsifier in food products , additives agent and anti-staling in bread industry [23] .

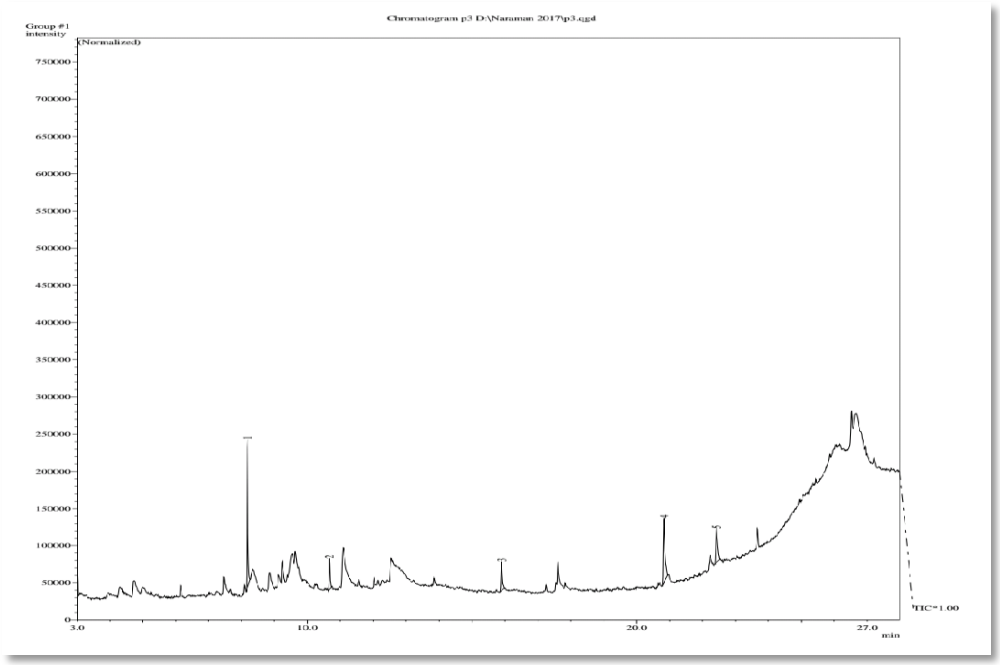

FIGURE 6. GC/MS diagram of Ms.

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TABLE 5. Ms Zahdi date seed composition

No	Identified compounds	Retention time (min)	Weight %
1	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8.180	39.18
2	3-[N'-(3H-Indol-3-ylmethylene)-hydrazino]-5-methyl-[1,2,4]triazol-4-ylamine	10.676	7.74
3	l-(+)-Ascorbic acid 2,6-dihexadecanoate	15.901	9.67
4	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	20.831	27.48
5	Octadecanoic acid, 2,3-dihydroxypropyl ester	22.422	15.92

- *Total Phenolic and Flavonoids Contents*

Total phenolic and flavonoids contents of Zs using water or ethanol or methanol as the extraction media at different concentrations are shown in figure 7,8 . Alcoholic extracts (Ethanolic and Methanolic) exhibited the highest content of phenolic and flvonoids compounds compared with water extracts (P<0.05). Concerning to phenolics was 67.32mg*l*ml for ethanolic extract . However the present results are much higher than those reported by [24] who found phenolic content in ethanolic extract of date seed 28.22 mg GAE/100g .The variation within may be owing to variety , growing condition , maturity , season , geographic origin ,fertilizer ,diseases soil type and storage conditions as well as extraction system as shown in the results of [25]. While the total content of flavonoids for aqueous extracts 33.32mg*l*ml and ethanolic extract 52.16 mg*l*ml and 46.16mg*l*ml for methanolic extract . Our results are in agreement with those reported by [26] who founded that the acetone (50%v/v) and ethanol(50%v/v) was used as extraction medium for date seeds , total phenolic and total flavonoid contents were higher , compared to that of water extract (P<0.05). Water exhibited poor ability to extract phenolics and flavonoids in the date seed due to the low solubility of these components in water [26] . Morever , protein and polysaccharides could be co-extracted when water was used alone for extraction . these substances might cause the fouling for filtration [27] .

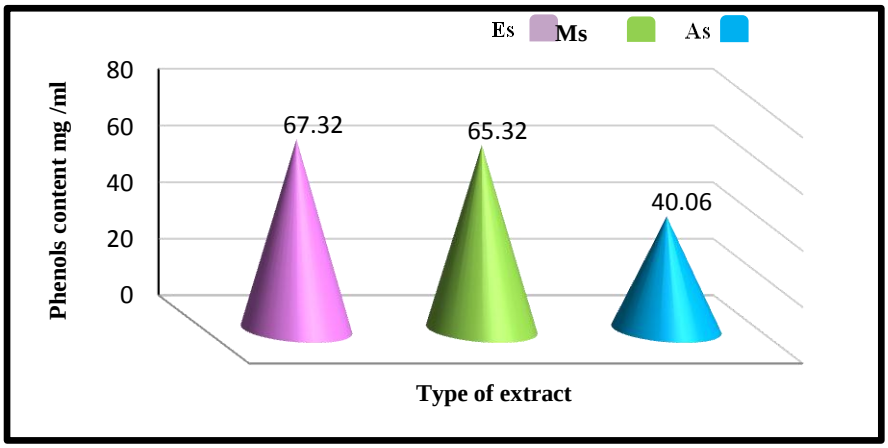

FIGURE 7. Total Phenolics in Zs extracts.

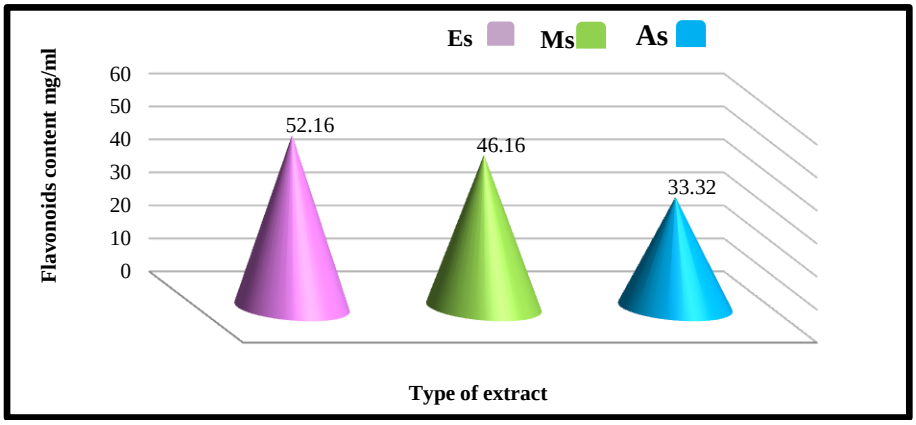

FIGURE 8. Total flavonoids in Zs extracts.

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- *Antioxidant Activity of Zs Extracts*

A-DPPH Assay

DPPH assay based on the ability of antioxidant to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical cations . DPPH radical scavenging activity of Zs extracts obtained from ethanol or methanol or water extraction at different concentrations and that compared with Butylated Hydroxy Toluene (BHT) are shown in Figure 9 . The activities increased when the concentration of extracts increased . An aqueous and alcoholic extracts of Zs exhibited antioxidant activity 88.70% for ethanolic extract and was ad equal to BHT activity 88.00% at 1.25mg/ml , and 87.02%for oil extract and 85.22%for methanolic extract but aqueous extract was the lowest 43.01% at the same concentration. The ability of the Zs extracts to scavenge DPPH radical was found to be related to the total phenolic and flavonoid contents . Our results are an agreement with those reported by [28] who founded that the antioxidant activity (EC_{50} mg/ml) of methanolic extracts obtained from two cultivars of date seed between 0.61-0.74 mg /ml for two types . DPPH radical scavenging activity of date seed extracts reflects their hydrogen donating ability [29] . In general, phenolic compounds capable of donating hydrogen atom are more effective in scavenging DPPH [30] .

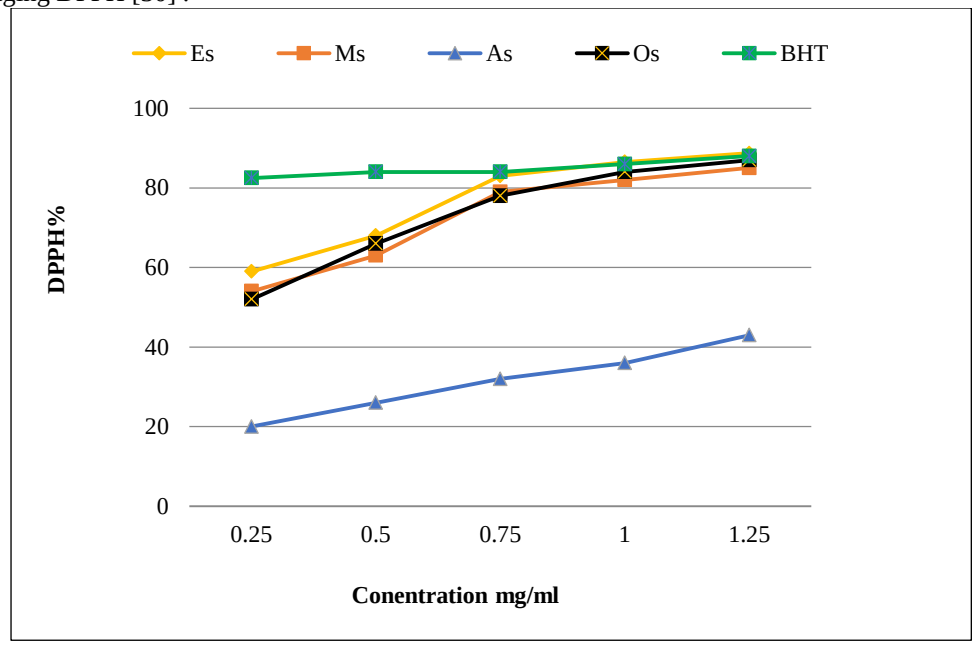

FIGURE 9. DPPH radical scavenging activity of Zs extracts.

B-Reducing Power

Reducing power is a measure of the ability of the extracts to reduce Fe^{+3} to Fe^{+2}. Substances which have reduction potential react with potassium ferri-cyanide (Fe^{+3}) to form potassium ferro-cyanide(Fe^{+2}) which then reacts with ferric chloride to form ferric ferrous complex that has an absorbance maximum at 700nm. The prepared extracts showed reducing power 2.63 for ethanolic extract and that was ad equal to alpha tocopherol , 2.342 for methanolic extract and 1.732 for oil extract and aqueous extract 0.993 at 1.25mg/ml (Figure 10) . Increase in reducing power of Zs extracts with increasing concentration of the extracts could be related to the increase in total phenolic and flavonoid contents . Date seed extracts has a relatively high reducing power . This might be due to the high antioxidant capacity of the seed extract because antioxidants are strong reducing agents and this is principally because of the redox properties of their hydroxyl groups and the relationships of any parts of their chemical structure [31] .

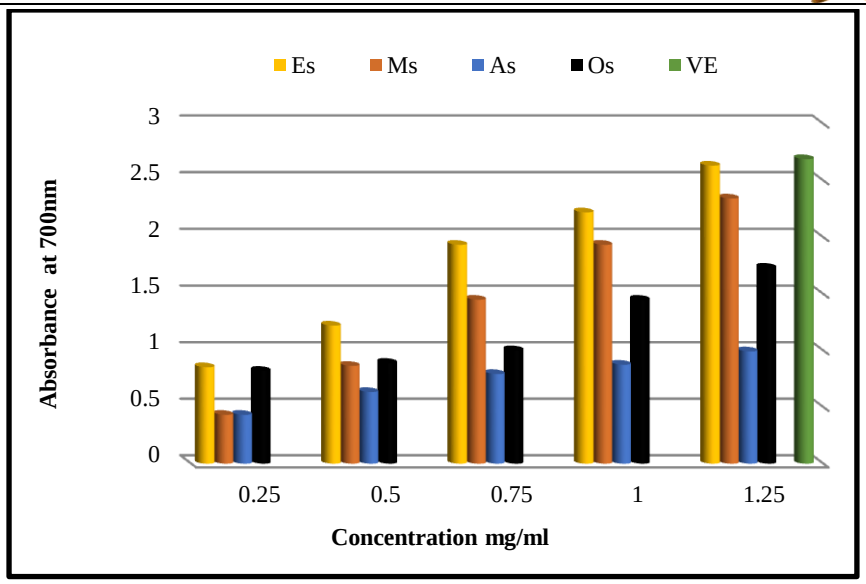

FIGURE 10. Reducing power of Zs extracts.

- *Chelating Ability of Ferrous Ion*

Chelating of iron ions activity of Zs obtained from ethanolic extract 60.93 % , methanolic extract 52.32% , oil extract 52.75% and aqueous extract 44.11% was compared with Ethylene Di-amine Tetra acetic acid Di-sodium (EDTA) 92.00% at 1.25 mg /ml is shown in Figure 11 . Zs extract obtained using ethanol or methanol higher metal chelating activity than that extracted with water ($P<0.05$) . Our results are an agreement with those reported by [32] who founded that the Date seed extract obtained from 20% (v/v) ethanol and 40% (v/v) acetone extraction showed the highest metal chelating activity . This could be due to the fact that phenolic compounds being extracted from the date seed could play the major role in metal chelating [33] .

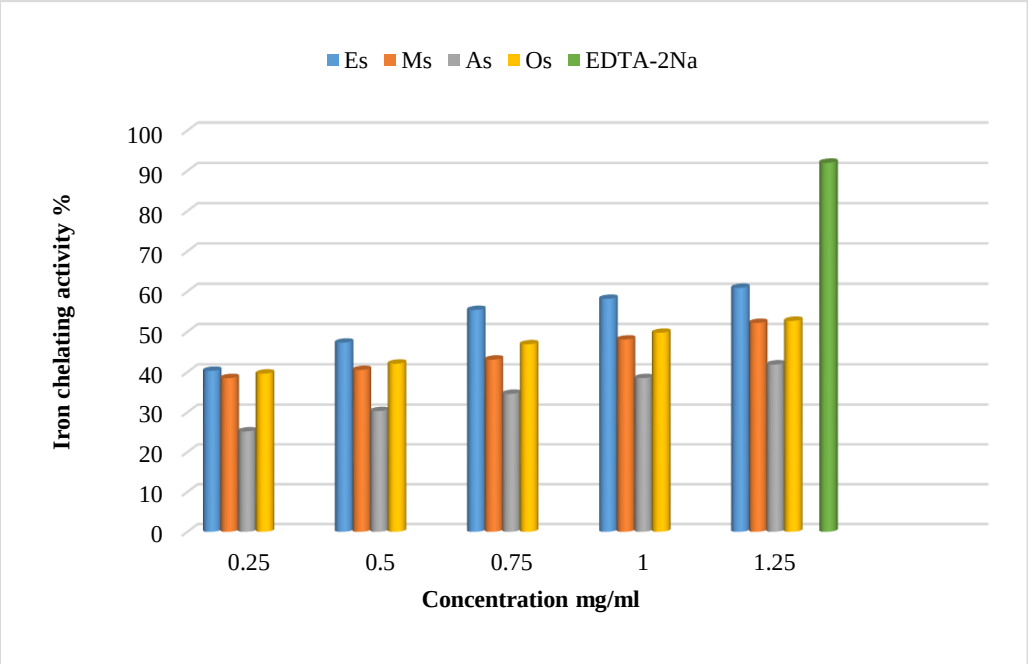

FIGURE 11. Chelating Ability of Zs Extracts.

- *Effect of Zs on Lipid Oxidation of Beef Patties*
- *Chemical Indicators*

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A-Peroxide Value (PV)

There was a significant decrease (P<0.05)in peroxide value in beef patties treated with ethanolic extracts of date seed compared with control that reach peroxide value to 5.87 meq*l*kg after 10-days of refrigerated storage . Impact of Es extract on lipid oxidation in beef patties is shown in Figure 12 . Also Es at higher concentration (0.1%) was more effective in lowering the formation of PV , compared with that with lower concentration (0.05%) . The results indicated that Es extract , especially Es at the concentration of 0.1% , effectively retarded lipid oxidation in beef patties . The ability of Es to retard lipid oxidation in beef patties might be caused by their phenolic and flavonoid contents and related with their ability to donate electron or hydrogen atom .

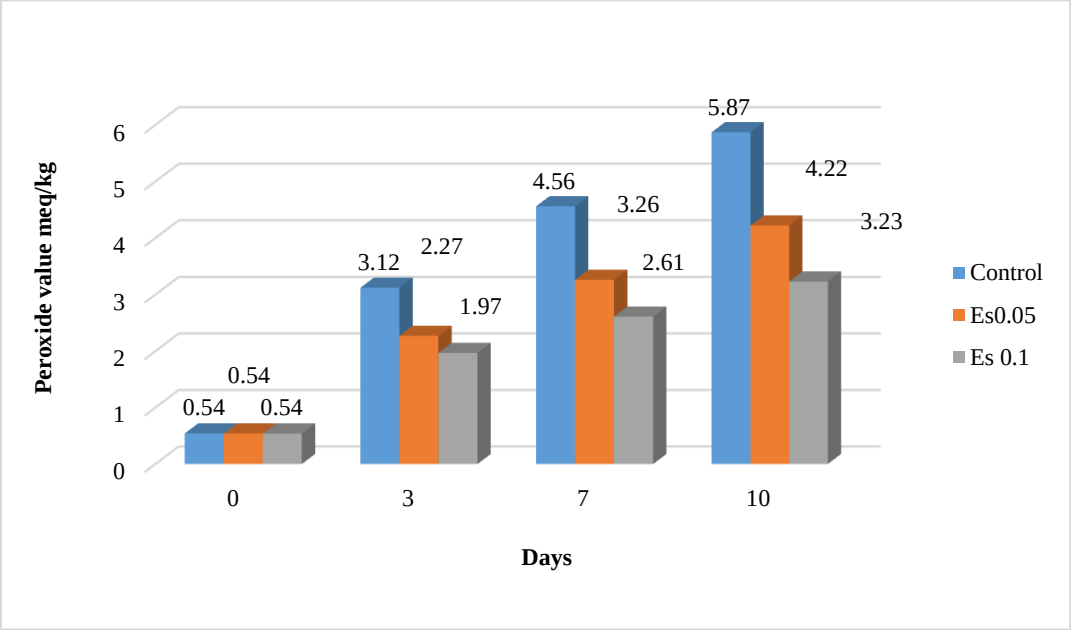

FIGURE 12. Impact of Es extract on PV value during 10 days of refrigerator storage.

B- Thiobarbituric Acid (TBA)

Impact of Es extract on TBA formation in beef patties during 10 days of refrigerated storage is shown in figure 13 . TBA values of control increased continuously during 10 days of refrigerated storage (*P*<0.05), which indicates that the control underwent lipid oxidation at a higher degree . When the beef was incorporated with Es , the TBA formation was inhibited greatly , compared to control (*P*<0.05) that reach to 3.93 malonaldehyde/kg after 10-days of refrigerated storage , indicating that Es could effectively retard lipid oxidation in beef patties . Extracts produced from different leaves and fruit seed were reported to have inhibitory effect on the formation of TBA in fish and meat model system [34-35] . Higher concentration (0.1%) of Es extract was more effective than lower concentration (0.05%) in retarding the formation of lipid oxidation products . The preventive effect of Es on lipid oxidation could be attributed to the presence of different phenolic compounds. Therefore , Es extract containing different phenolic compounds could be a promising antioxidant and play an important role in preventing lipid oxidation in beef patties .

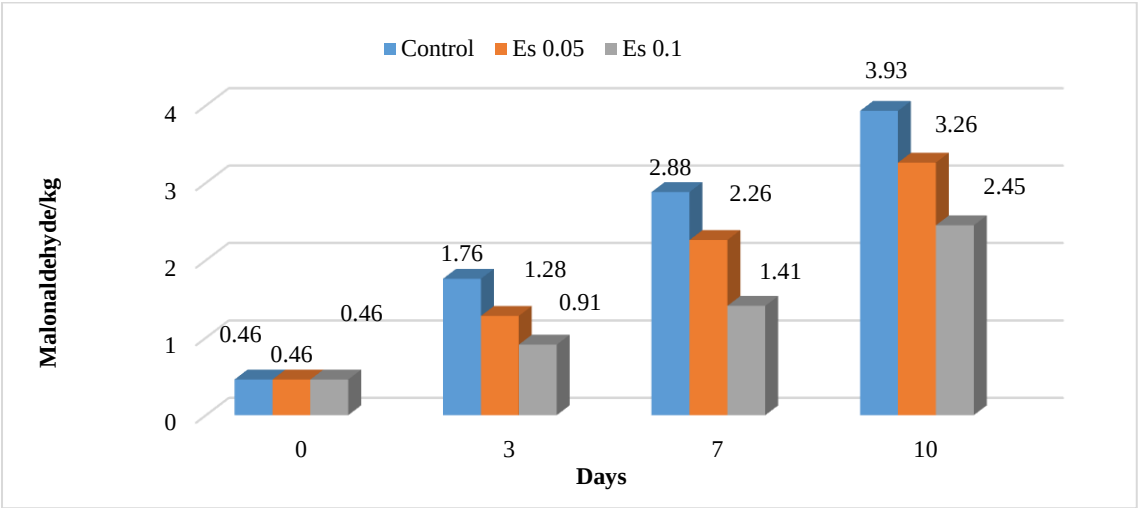

FIGURE 13. Impact of Es extract on TBA value during 10 days of refrigerator storage.

CONCLUSION

The high amounts of phenolics , flavonoids , antioxidants and the good proximate composition require a high valorisation of this by-product using it as ingredient to enhance the nutritional value of some functional foods for human and animals consumption .

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