

## Antioxidant activity and phenolic contents of Ajwa date and their effect on lipo-protein profile

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### ABSTRACT

**Background:** Dates (*Phoenix dactylifera L.*) are well known as both a food and economic crop for many years worldwide due to its substantial nutritional, health, and economic benefits besides its appeal and environmental value. However, although Date pits are rich in phenolic and antioxidant contents, they are generally neglected and treated as a waste product. Ethnobotanical records indicate the potential of Ajwa dates pits and flesh having phenolics, antioxidants, and some other nutrients.

**Objective:** The purpose of the study was to extract the phenolic contents and to determine the antioxidant scavenging activity of Ajwa date flesh and pits in comparison to local date varieties in three different solvents with three different concentrations, and to observe their effect on the high lipo-protein profile of albino mice.

**Materials and Methods:** Three solvents viz. Methanol, Ethanol, and Acetone were used at concentrations of 70%, 80%, and 90% to make nutraceutical extracts. These extracts were characterized for numerous nutritional parameters. Correlation of flavonoid with phenolic, DPPH, ABTS, and other parameters were studied. The above extracted Ajwa flesh and pits extracts were then administrated to high cholesterol induced albino mice under three different treatments (10%, 20% and 30%) in four different groups where one group was studied as control

**Results:** Pits appeared to be the richest source for total phenols and total flavonoid contents. Such extracts from pits were high in DPPH and ABTS activity compared to that of the flesh in all date cultivars, which were the highest in Ajwa pits (3932.3 mg GAE/100g, 2956.2 mg QEC/100g,

96.3% and 86.2% respectively). In the comparative analysis of solvents, 80% ethanol extracted the highest antioxidant activity for both DPPH and ABTS assay in all date varieties and their parts. Behaviour of solvent for polyphenol (Total phenols and total flavonoids) extraction was highly variable so that a single solvent cannot be recommended for extraction. Highly significant correlation of flavonoids with DPPH was observed in pits part. Phenolic contents positively correlated with flavonoids, DPPH, and ABTS. DPPH and ABTS also revealed a strongly positive correlation. In PCA analysis phenolic contents, flavonoid contents and antioxidant activity showed a strong relation among pits, while demonstrating a weak relation in flesh parts. Furthermore, results also depicted that 30% extracts of Ajwa pits have a higher effect on the percent reduction of total protein, total triglycerides, total cholesterol, and LDL-C levels, in addition to a significant percent increase in HDL-C levels in treated albino mice

**Conclusion:** The work identifies variability among the flesh and pits of Ajwa date and local date varieties for their polyphenols contents, antioxidant activity, and their health beneficial effect against a high Lipo-protein profile that can be used as an active ingredient against different maladies in food products and supplements.

**Keywords:** PCA, Ajwa, lipo-protein, phenolics

## INTRODUCTION

Dates (*Phoenix dactylifera*) are the oldest cultivated plant in the world and renown for its substantial nutritional, health, and economic benefits, in addition to its aesthetic and biological importance. In addition to their nutritional properties, dates are believed to have medicinal properties due to the presence of different bioactive compounds. The presence of natural antioxidants and phenolics in dates make them suitable against different maladies like infectious and bacterial diseases, diabetes, hyperlipidaemia, and cancer. However, these phenolic and antioxidant components may differ in date varieties and their parts [1; 2]. The differences in the quantity of these antioxidants depend on many factors including, solvents, variety, and parts of date fruit. Among these factors, the solvent type is important to quantify the phenolic and antioxidant contents, as different solvents with different concentrations may have an effect on the quantity of these antioxidants [3]. Similarly, another important factor which can affect nutritional and medicinal value is variety. Among all date varieties, the Ajwa date can be distinguished due to the higher nutritional and medicinal properties it contains. In addition to the variety of dates, different date parts within the same variety have different composition of nutrients and bioactive components [4]. There are documented ethano-pharmacological records indicating the potential of date flesh and pits against hyperlipidaemic problems and several other ailments. However, there are less experimental results on the effects of Ajwa date parts against hyperlipidaemia. This demands a detailed scientific study to explore total phenolics and the antioxidant activity of Ajwa dates in comparison to other varieties. From a public health point of view, one of the major factors contributing to the development of cardiovascular diseases is hyperlipidaemia [4].

Until recently, several studies have demonstrated that controlling hyperlipidaemia results in significant reduction in death rate due to stroke and other heart diseases. With this scientific need in mind, the purpose of the study was to extract phenolic contents of Ajwa date parts (flesh and pit) in different solvents and their concentrations, in addition to also determining their antioxidant

scavenging activity in comparison to local date varieties. Finally, the potential of the extract for having the best profile for phenolic contents and antioxidant scavenging activity was evaluated to study parameters of hyper lipo-protein profile in albino-mice.

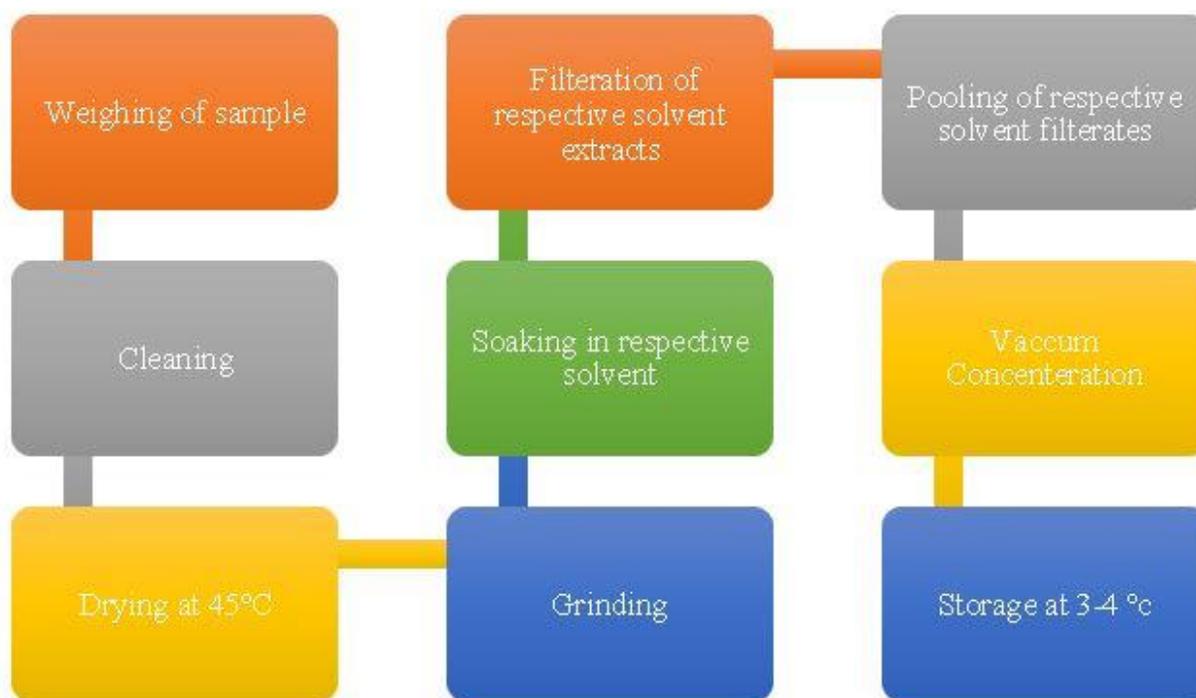
## MATERIALS AND METHODS

### *Preparation of Samples*

The Ajwa date samples were purchased from renowned stores of Rawalpindi, Pakistan and were botanically identified from Department of Botany, PMAS-Arid Agriculture University, Rawalpindi while local date varieties (Aseel and Zaidy) were bought from Date palm research centre, Jhang, Punjab, Pakistan. Fully developed fruits of uniform size, free of fungal infection and any physical injury were selected, washed with distilled water, and then dried. Date pits were grinded with a heavy duty grinder and all samples were stored at ambient temperature until used for further analysis.

### *Preparation of Extracts*

Date flesh and pits samples were extracted separately using three solvents (methanol, ethanol, and acetone) in three different concentrations (70%, 80%, and 90%) for each solvent as explained in Figure 1.



**Figure 1.** Schematic diagram for extraction in different solvents

### *Total Phenolic Contents (TPC)*

The total phenolic content of both flesh and pits of all date samples were determined with the Folin- Ciocalteiu reagent method of Benzie and Strain [5]. The concentration of total phenolic contents in extracts was expressed as mg Gallic acid equivalent (GAE) per 100 g.

**Total Flavonoid Contents (TFC)**

Total flavonoid contents in all date samples were determined by DPPH assay according to the method of Kim et al [6]. The concentration of total flavonoid contents in extracts was expressed as mg QEC equivalent (QEC) per 100 g.

**DPPH Scavenging activity**

DPPH solution was prepared for determination of antioxidant activity in date samples according to the method of Brand-Williams et al. [7]. Stock solutions of DPPH were prepared by adding 0.2 g of DPPH in in 50 ml of respective solvents (methanol, ethanol, and acetone). Similarly, stock samples in their respective solvent were prepared containing 1mg extract/ml of solvent. 1 ml from these respective stock solutions were added to the respective test tubes labelled with their name. 2 ml from respective DPPH stock solution was added to each test tube. In the same way, control solutions were also prepared containing 2 ml of DPPH and 1ml of Gallic acid solution. All test tubes were incubated at room temperature in the dark for 30 minutes. The decrease in absorption of extract mixtures and control was recorded at 517 nm and was measured using spectrophotometer. Percent inhibition of DPPH radicals by samples and control was calculated using the following formula:

$$\text{DPPH (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where  $A_0$  = absorbance of control and  $A_1$  = absorbance of sample extract

**ABTS Scavenging Activity**

The ABTS assay is based on the capacity of a sample to scavenge the ABTS radical cation (ABTS) compared to a standard antioxidant (Quercetin), by adopting the method of Arnao et al. [8]. The ABTS solution was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration) for 12-16 h, in the dark, at room temperature. Prior to use, the ABTS working solution was prepared by diluting the stock solution with EtOH to an absorbance of  $0.70 \pm 0.02$  at 734 nm. The samples and Quercetin standards (20  $\mu$ l) were combined with the ABTS working solution (170  $\mu$ l, absorbance  $0.70 \pm 0.02$ ). After 6 min of incubation at 30°C, the absorbance at 734 nm was measured. ABTS scavenging activity for all samples was expressed as:

$$\text{ABTS Scavenging(\%)} = \frac{A_0 - A}{A_0} \times 100 \text{ Where, } A_0 = \text{Absorbance of control and } A = \text{Absorbance of sample}$$

The extract of date parts studied with the best profile of phytochemicals and their antioxidant activities were further studied for their efficacy against induced hypo cholesterol in animal models.

**EFFECT ON LIPO-PROTEIN PROFILE****Hyper cholesterol induction to animals**

In the second experiment, the effect of the date extract with the best profile of bioactive components on the lipoprotein profile were studied in comparison with local date varieties. Higher cholesterol levels were induced by feeding mice feed mixed with cholesterol and casein powder. Serum samples of some mice were tested to ensure hyper cholesterol induction in the animals.

**Animals and experimental protocol**

During the study period, the experimental animals (albino mice) of uniform age (4 weeks old) were given a diet mixed with date extracts and water ad libitum. Male and female animals were kept in separate animal houses under same conditions (12 hrs of light/dark,  $24 \pm 2^\circ\text{C}$  temperature,

and 65% humidity) and provided with treatments. The study was carried out on four groups of mice (10 mice per group, 5 males and 5 females). One group (male and female) was studied as the control group, while the other three groups of mice were studied under date pit extracts of the best profile. After the end of the experiment, period blood samples from the animals in all groups was taken under light ether anaesthesia with a fasting period of 18 hours. Blood samples from all animals were taken for further analysis. Data of all parameters was presented as percent change in comparison to control group data.

Experiments were performed as per ethics and permission of Pakistan veterinary medical council.

### ***Serum separation***

Serum samples were prepared by collecting mice blood after sacrificing in Eppendorf tubes containing 0.8 ul EDTA and were mixed gently, which was followed by centrifuging blood at 5000 rpm for 20 minutes and were stored at low temperature (-20° C) for further analysis.

The following analysis were conducted:

### ***Determination of total proteins***

Total proteins for all serum samples were assessed as per method of Gadder, 1996 [9]. Briefly, three test tubes labelled as sample, standard, and blank were taken. In these tubes, 20 µl sample, 20 µl distilled water and 1000 µl reagent 1 were added. All solutions were mixed well and incubated at 20-25°C for 5 min., before the absorbance (A1) was read at 540 nm against the blank. Afterwards, 250 µl of reagent 2 was added to both tubes and incubated again at 20-25°C for 5 min. Finally, absorbance (A2) was measured and change in absorbance calculated according to the formula:

$$\Delta A = [(A2-A1)] \text{ sample} - [(A2-A1)] \text{ blank}$$

Then, two test tubes labelled as sample and blank were taken. In these test tubes, 20 µl sample and 20 µl distilled water were added respectively, followed by the mixing of 1000 ml of mono-reagent in both tubes. After mixing well, test tubes were nurtured at 37°C for 5 min and then absorbance was measured at 540 nm. Total proteins were then calculated according to the following formula:

$$\Delta A = A (\text{sample}) - A (\text{blank})$$

$$\text{Total protein} \left( \frac{\text{g}}{\text{dl}} \right) = \frac{\Delta A (\text{Sample})}{\Delta A (\text{Standard})} \times \text{conc of standard}$$

### ***Determination of total triglycerides***

Total triglycerides were determined according to the procedure of Natio, 2003 [10]. Briefly, 1000µl of reagent and 10µl of sample was taken and well mixed followed by incubation for 10 minutes at 20°C ± 3°C for 5 minutes. Afterwards, absorbance of sample (As) resp. of the standard (Ast) was measured against the reagent blank. The absorbance remains stable for 1 hour. Total triglycerides were calculated as:

$$\text{Triglycerides concentration} = A_s \times \frac{\text{Conc. of standard}}{A_{st}}$$

***Determination of total cholesterol***

Total cholesterol was determined by the method discussed by Natio, 2003 [10]. Three test tubes were taken labelled as sample, standard, and blank. In each test tube, 10 µl of the sample, standard, and distilled water was added, followed by the addition of 1000 µl of reaction solution to each test tube, and then incubation for 10 minutes at 20 °C- 25 °C. Absorbance of sample (As) and standard (Ast) was measured against the reagent blank at 500 nm. The absorbance remains stable for 45 minutes (Natio, 2003). Concentration of total cholesterol was calculated as:

$$\text{Concentration of cholesterol} = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{Std}}} \times \text{Conc Std. [mg/dl]}$$

***Determination of HDL-C***

High density lipoprotein-cholesterol for all serum samples were calculated by the following method of Natio, 2003 [10]. Three test tubes labelled as blank, standard, and sample were taken. In the blank test tube, 2.4 µl of distilled water, 2.4 µl of standard, and 2.4 µl of sample were added. Afterwards, in all three test tubes, 240 µl of reagent R1 was added before being well mixed and incubated at 37°C for 4 min. 40 sec. Absorbance of sample (A1) and standard (As1) were measured, then added to 80 µl of reagent R2 in blank and sample test tubes. Next, we mixed them well and incubated the test tubes again for 4 minutes and measured the absorbance of sample (A2) and standard (As2). HDL was calculated as:

$$\text{Concentration of HDL} = \frac{A2 - A1 (\text{sample})}{As1 - As2 (\text{standard})} \times \text{Conc of standard}$$

***Determination of LDL- C***

Low density lipoprotein-cholesterol for all serum samples were calculated by following the method of Sinha [11]. Same procedure was followed, taking three test tubes labelled as sample, blank and standard. 30µl of sample, blank, and standard was followed by the addition of 300µl of reaction solution added to each test tube. Test tubes were mixed well and then incubated at thirty-seven degree centigrade for five minutes. The absorbance was measured as the following: first absorbance of sample (A1) and standard (As1) were measured against blank at 600 nm; then 90 µl of reagent B was added in all three test tubes and absorbance of standard (As2) and sample (As1) was measured again; concentration of LDL was calculated by the formula:

$$\text{Concentration of LDL} = \frac{A2 - A1 (\text{sample})}{As1 - As2 (\text{standard})} \times \text{Conc of standard}$$

***Statistical Analysis***

Principal component analysis, correlation and Scatterplot analysis were used to observe relationships within various parameters. For all statistical analysis, R studio and Minitab version 16 soft wares were used.

**RESULTS AND DISCUSSION*****Total phenolic contents***

Phenolic contents of flesh and pit of different date varieties fluctuate widely in each type of solvent concentration. Interaction between solvent, concentration, varieties, and part of date (flesh or pits) have a significant effect on TPC ( $p > 0.05$ ). Results showed that among all of the date varieties, Ajwa date had higher amounts of TPC, while pits of all date varieties have significantly higher total phenolic contents compared to the flesh parts. Similarly, among all solvents and their

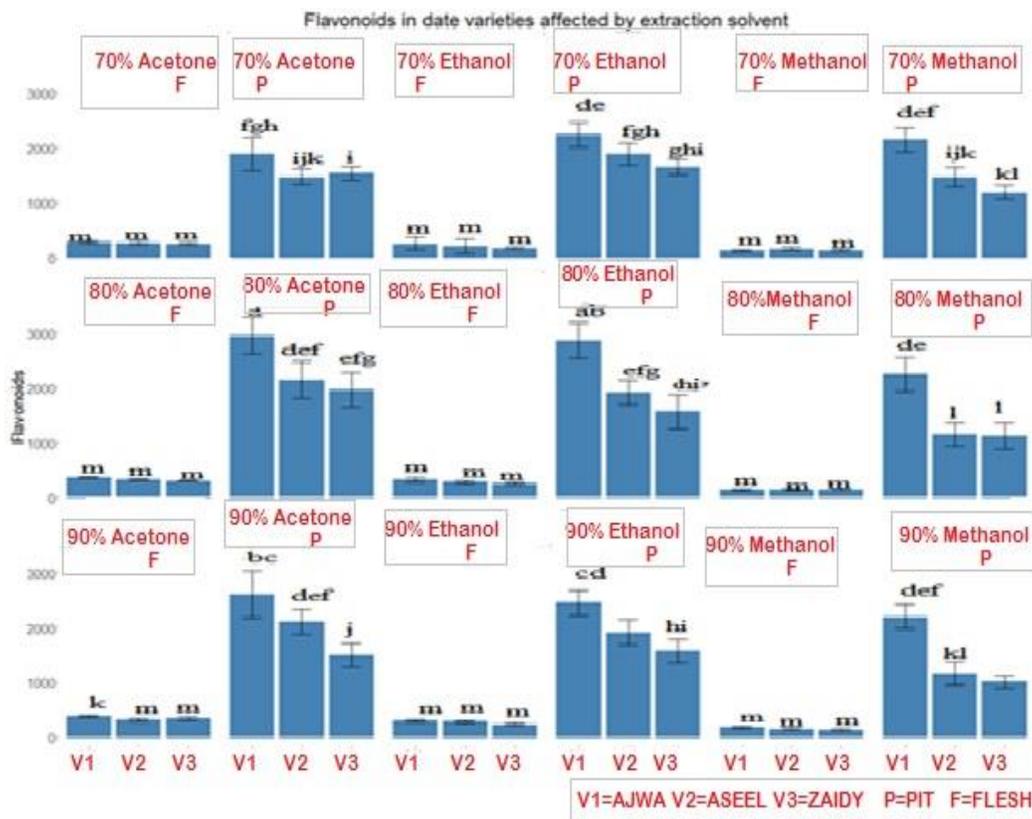
concentrations, 90% ethanol was found to be the most effective to extract higher amounts of TPC in all date varieties and their parts (Figure 2). Results further showed that among all date varieties and their parts, Ajwa pits had a range of phenolic contents between 3932.3 mg GAE/100g (90% ethanol) to 3154.7 mg GAE/100g (70% ethanol), while Zaidy pits had a lower range of total phenols from 2432.2 mg GAE/100g (90% ethanol) to 2333.5 mg GAE/100g (70% ethanol). Furthermore, variable trends for TPC extraction in different solvents and their concentrations is an evident depiction that in some parts TPC can be extracted using 90% ethanol, while in other parts TPC can be extracted through 80% ethanol, 70% ethanol, and 90% acetone. Results suggest that solvent polarity plays a basic role in enhancing phenolic solubility [3] and that water-organic solvent mixtures are more effective towards the extraction of total phenolic contents. Therefore, it is difficult to develop a standard extraction procedure that will be appropriate for the extraction of phenolic contents of all plants and their parts [3]. Furthermore, results suggest that pits have remarkable potential for polyphenols compared to that of the flesh, so date pits can be a better source of phenolic contents than flesh parts of date cultivars irrespective of variety and solvent used. Differences in flesh and pits quantities may be due to their physiology and chemical constituents. However, due to their higher potential of phenols, these can be used as potential source of phenolics and antioxidants in food products [1; 12]. The results further suggest that more work on the isolation of these phenols derived from date parts is required so that synthetic phenols can be replaced in foods.



**Figure 2.** Total phenolic contents in date varieties and parts in different solvents and their different concentration

**Total flavonoid contents (TFC)**

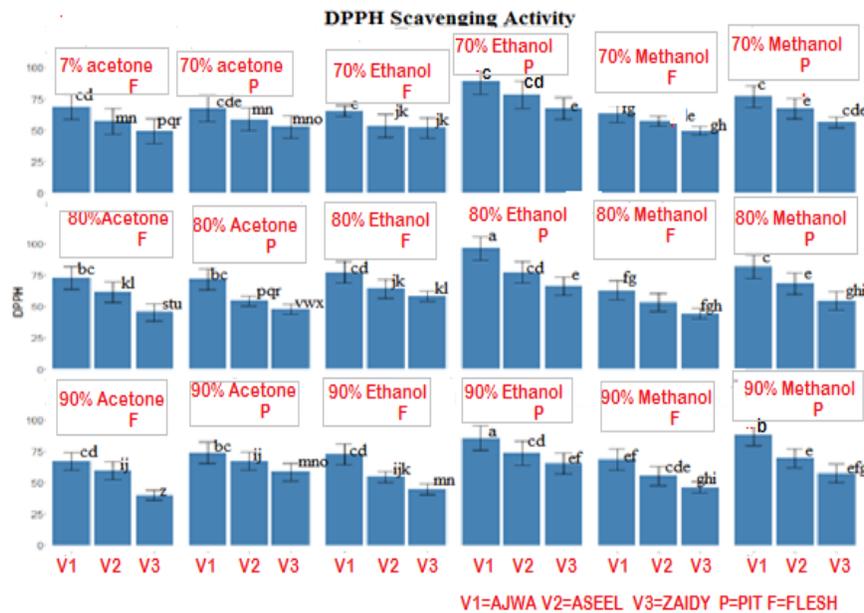
Results showed that among all date varieties, Ajwa date had a higher amounts of TFC, whereas pits of all date varieties have significantly higher total flavonoid contents compared to that found within the flesh parts of the dates. Similarly, among all solvents and their concentrations, 90% ethanol was found to be the most effective in extracting higher amounts of TFC in all date varieties and their parts (Figure 3). However, the flesh and pits of date cultivars also revealed extensive differences for flavonoids contents in all varieties (Figure 3). In contrast, Ajwa pits highest quantity of TFC ranged from 1897.4 mg QEC/100g (70% acetone) to 2956.2 mg QEC/100g (80% acetone), while zaidy pits have the least amount of flavonoids in a range between 1013.6 mg QEC/100 g (90% methanol) - 1962.3 mg QEC/100g (80% actone). The significant effect of solvent and its different concentrations is also evident on flavonoids. These differences may be due to variable polarity of the solvents which plays a basic role in enhancing flavonoids solubility [3]. Results further suggest that 80% acetone was the best solvent to extract flavonoids from both parts of all date cultivars, recommending it as the best solvent to extract flavonoid contents which may be due to its higher capacity to dissolve polar flavonoids. Overall, pits of date varieties are better sources of flavonoids than flesh. The variation in flavonoid contents of flesh and pits may be due to their genetic makeup, solvent used, and solvent concentration, which suggests there should be more studies on date pits to explore their flavonoids and to also make it possible to use them in food industry to minimize their wastage. Further studies are also recommended for extraction of non-flavonoids contents from date parts and cultivars.



**Figure 3.** Total flavonoids contents (mg GAE/100g) in date varieties and parts in different solvents and their different concentrations

**Total antioxidant activity**

Different methods are employed for evaluation of antioxidant activity in fruits, of which ABTS and DPPH are most common. In this study, the antioxidant activity of date flesh and pits were evaluated by two methods viz. DPPH and ABTS. Moreover, antioxidant activity is affected by solvent type, concentration, varieties, and their different parts. For DPPH assay, interaction between solvent, concentrations, variety, and parts have significant effects on DPPH scavenging activity of date parts ( $p>0.05$ ) (Figure 4). Generally, among all date varieties, Ajwa dates have higher amounts of DPPH scavenging activity, whereas pits of all date varieties have significantly higher amounts of DPPH scavenging activity compared to the flesh parts of dates. Similarly, among all solvents and their concentrations, 90% ethanol was found most effective in extracting higher amounts of DPPH scavenging activity in all date varieties and their parts (Figure 4). Among pits, Ajwa pits exhibited the highest DPPH scavenging activity range between 96.3% (80% ethanol) to 67.3% (70% acetone), while Zaidy pits had the lowest DPPH scavenging activity varying from 67.1% (70% ethanol) to 47.6% (90% methanol) in different solvents (Figure 4). Similarly, in ABTS assay solvent, interaction between variety, concentrations of solvent, solvent and parts had significant effects on ABTS scavenging activity ( $p> 0.05$ ). Normally, date pits significantly showed more ABTS scavenging activity in comparison to date flesh of all varieties (Figure 5). Within parts, Ajwa pits have highest amount of ABTS scavenging activity in range of 86.2% to 69.3% followed by Zaidy pits which have ABTS scavenging activity varying from 65.9% to 54.2% (Figure 5). Results suggest that the antioxidant activity of different extracts has strong association with different concentrations of solvent used, mainly due to different scavenging capacity of compounds with different polarities [14]. Therefore, it can be suggested that antioxidants are more dissolved in polar solvents and that among them ethanol and its water concentrations (80% and 70%) are the most effective solvent for extraction of date antioxidants, due to a wide range of compounds that it can dissolve. Differences among pits and date flesh recommend pits to be a greater source for antioxidants, which may be due to their higher phenolic and flavonoid contents. However, further studies are recommended for the isolation of these antioxidants.



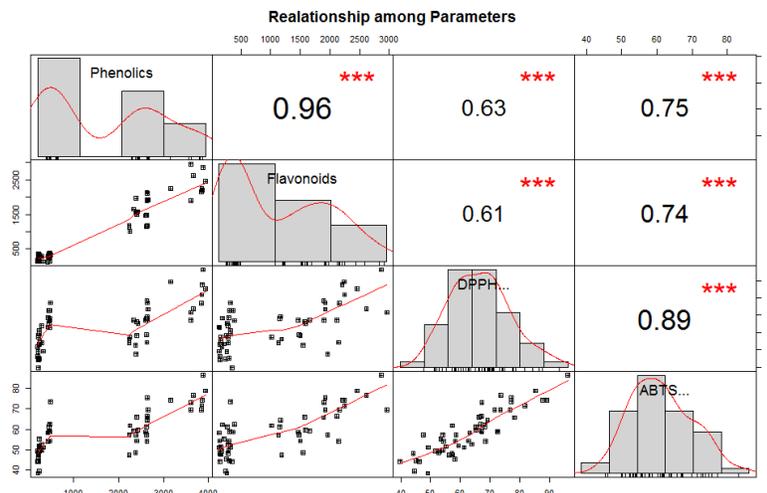
**Figure 4.** DPPH scavenging activity (%) in date varieties and parts in different solvents and their different concentrations



**Figure 5.** ABTS scavenging activity (%) in date varieties and parts in different solvents and their different concentrations

**Relationship analysis**

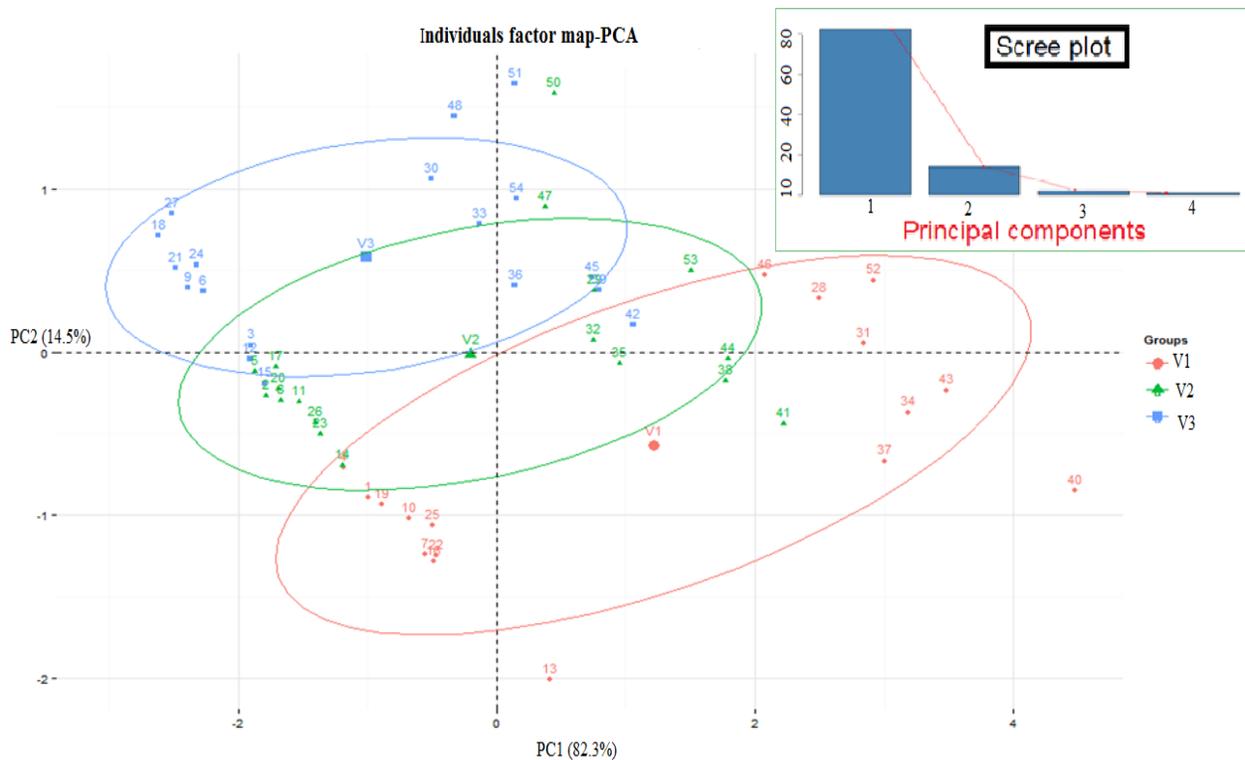
Irrespective of date parts and solvents, the histogram shows that most of date cultivars have phenolic contents ranged between 200-1000 mg GAE/100g, flavonoids between 300-1200 mg QEC/100 g and DPPH and ABTS contents between 55-65% (Figure 6). Phenolic contents have a significantly higher positive correlation with flavonoids (0.96), DPPH (0.63), and ABTS (0.75). Similarly, a strongly positive correlation was observed between flavonoids, DPPH (0.61), and ABTS (0.74), while DPPH and ABTS (0.89) were also revealed to have a strong positive increasing relationship, as evident in Figure 4. Results suggest that phenolic and flavonoid contents have a strong effect on antioxidant activity of date fruits. So varieties could be nominated by relying on one of the above stated attributes for these parameters while studying their antioxidant properties.



**Figure 6.** Relationship between phenolic, flavonoids and antioxidant activity

**Principle component analysis**

Significantly, new plant sources for natural antioxidant are being used to replace synthetic antioxidants. Many literature reports designate studies of antioxidants from date palm and their parts. To understand more about variables and variation between date parts, PCA was applied based on their antioxidant, phenolic, and flavonoids contents. The scatter plot was used to classify the number of principle components (Figure 7). Eigen value for first two components dropped sharply while remaining stagnant for others with a cumulative variation of 96.8%, so we will take the first two components for study. Bi-plot shows variability among three varieties on the basis of their variables in two dimensions: V1 is highly apart from V2 and V3, while V2 and V3 are overlapping (Figure 7). The leading PC1 explains variability of 82.3% and is highly contributed by variables of V1 (Ajwa). while PC2 explains 14.6% variability for the variables of V2 and V3. In particular, PC1 is highly correlated with phenolics, flavonoids, and DPPH and ABTS activity of V1, while PC2 is positively correlated with phenolic, flavonoids, and DPPH and ABTS contents of V2 (aseel) and V3 (zaidy). This technique serves as a valuable tool for understanding much more about the explanation of results from the research study and effects of date varieties on them. Assessment of phenolics, flavonoids, and antioxidants in each variety can assist in the classification of date cultivars.



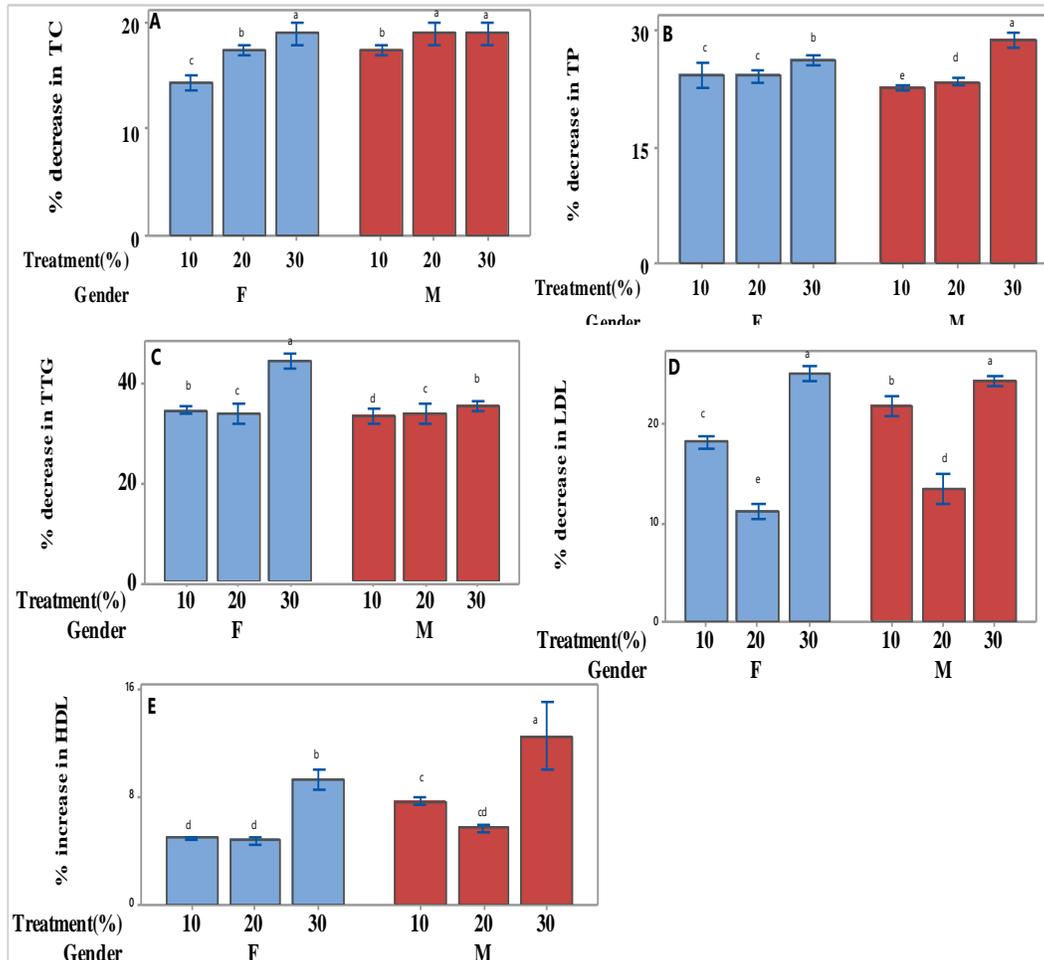
**Figure 7.** PCA plot for data set of date varieties

**Effect on lipoprotein**

In this assay, the effect of Ajwa date pit extracts dissolved in 90% ethanol were evaluated against higher lipoprotein profile in both male and female albino mice. The results revealed that among the three different treatments of Ajwa pits extracts, 30% dose of extracts was most effective in

reducing percent level of total cholesterol in both male (18.4%) and female (19.2%) mice significantly, while 10% dose was least effective in both genders (16.8%; 14.1% respectively) (Figure 8 A). The same trend was also observed for percent decrease in total protein contents, where also 30% dose of the Ajwa pits extracts was most effective in both male (28.5%) and female (25.7%) albino mice, while 10% dose was least effective in both genders (23.5%; 23.9% respectively) (Figure 8B). Furthermore, results also revealed that 30% dose of Ajwa pits was most effective for the percent decrease in total triglycerides levels in both male (35.1%) and female mice (44.5%), while 20% dose of Ajwa pits were least effective for the percent decrease in TTG levels in male mice and 10% dose being the least effective in female mice (Figure 8C). The same trend was also observed in percent decrease in LDL levels where 30% dose of Ajwa pit extracts was most effective respectively in male and female mice (32.5%). Likewise, results also revealed that in male mice 20% dose of ajwa pits was least effective on percent decrease in serum LDL in both male (13.5%) and female (10.9%) groups (Figure 8D). Epidemiological studies suggest that there is positive correlation between protein consumption and CHD ( $r=0.78$ ) because these protein levels are the primary source of cholesterol and saturated fats which increases risk of CHD (Figure 8). Our results suggest that Ajwa pit extracts can significantly reduce serum total protein percentages in cholesterol fed male and female mice, which may be due to their high enrichment in phenols and flavonoids. Several studies have revealed the protective effect of polyphenols on coronary heart diseases [14] by reducing the oxidative changes in lipoprotein fractions. The significantly higher effects of 30% dose may be due to the higher number of polyphenols than other treatments. Moreover, the differences between genders can be due to their endocrinological and physiological factors. A strong relationship exists between triglycerides levels and cardiovascular diseases. Triglycerides are the most problematic lipid, usually measured in the evaluation of CVD risk, which is due to its strongly inverse relationship with HDL. It lowers levels of HDL and LDL-C, which increases risk of CVD [15;16]. The results of this study demonstrated a promising effect of 30% extracts of Ajwa pits in significantly reducing serum triglycerides level in mice. While among genders female mice have higher percentages of reducing triglycerides, which may be due to their ability to survive in environment provided and may also be due to the presence of elevated levels of estrogenic hormone, which is linked with reduction of lipids and cholesterol in blood. Similarly, results also showed that in both male (10.2%) and female (8.9%) groups, 30% was more effective for percent increase in serum HDL levels while 20% dose was least effective in both male (5.0%) and female (4.9%) groups (Figure 8D). Oxidative damages are main reasons for atherosclerosis, which is mainly due to modification of LDL for scavenging receptor uptake causing accumulation of cholesterol in cells. The antioxidants in Ajwa pits inhibited peroxidation of LDL and reduced risk of atherosclerosis in hyperlipidaemic in both male and female mice [17]. Furthermore, it is suggested that LDL is generally oxidized by metal ions or muscle cells, endothelial cells, and macrophages which usually produce free radicals that causes oxidation of LDL; thus, a defensive system against these free radicals is required that can prevent LDL from oxidation [18; 19]. These results suggest that administrating 30% Ajwa date pits can prevent LDL oxidation which can also protect against atherosclerotic progression. Recent studies have suggested that HDL can promote the reverse cholesterol transport pathway. This pathway includes that HDL can induce efflux of excessive accumulated cholesterol protecting the production of oxidative modified LDL [19; 20]. The results stated above suggest that Ajwa pit

extracts have positive effects on inhibition of LDL modification and also promote the excessive efflux of accumulated cholesterol from cells by elevating serum HDL levels [21; 22]. The results further suggest that 30% dose of Ajwa pit extracts have hypocholesterolaemia effect on both genders on mice but on male mice the effect was significantly higher, which may be due to endocrinology and physiological effect.



**Figure 8.** Effect of Ajwa pits treatments on Lipo-protein profile

**CONCLUSION**

Comparative analysis between date varieties and parts (flesh and pit) showed higher quantities of total phenolic, total flavonoids, and antioxidant contents in date pits than flesh in 90% ethanol, while Ajwa pits were found to be the richest source of all of the parameters described above in both parts of all date varieties. In correlation analysis, phenolic contents have a significant positive correlation with flavonoids and DPPH and ABTS. Similarly, a strongly positive correlation was also observed between flavonoids and DPPH and ABTS, while DPPH and ABTS also revealed a strongly positive increasing relationship. Based on PCA analysis, the data group was clearly divided into two subsets. Pits were separated from flesh parts based on their phenolic contents, flavonoid contents, and antioxidant activity, which will be helpful in the classification of date parts and cultivars. Furthermore, Ajwa pits were evaluated for their biological effect against lipo-protein profile, concluding that 30% extract of Ajwa pit was effective against percent decrease in total

protein, total cholesterol, total triglycerides, and LDL levels, and were also effective for percent increase in HDL levels. Thus, higher phenolic and antioxidant components in Ajwa date pits emphasize their use in food and pharmaceutical industry as functional and disease protective ingredient in food products and supplements

**List of Abbreviations:** TPC, Total phenolic contents; TFC, total flavonoid contents; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid; HDL, High density lipo-protein; LDL, Low density lipoproteins; TP, Total proteins; TTG, Total triglycerides; TC, Total cholesterol.

**Authors' contribution:** All authors have equally contributed in this work.

**Competing Interests:** Authors have no conflict of interest.

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