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IN VITRO EVALUATION OF *P. DACTYLIFERA* AND *J. REGIA* NUTRACEUTICAL EXTRACTS THROUGH ANTIOXIDANT ASSAYS, CYTOTOXIC STUDY AND THEIR EFFECT ON SPERM MOTILITY

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Abstract

The present research work was being planned to explore the nutritional and antioxidant potential of *P. dactylifera* and *J. regia* hydromethanolic extracts. Proximate analysis of *P. dactylifera* fruit showed that it was rich in dietary fibers, minerals, vitamins, sugars. Proximate analysis of *J. regia* kernels showed that it was rich in fats and proteins. Qualitative and quantitative phytochemical analysis revealed the presence of phenolics, flavonoids and glycosides in both nutraceutical extracts. *In vitro* antioxidant profiling revealed that both extracts can significantly scavenge DPPH free radical and they have a potential to potential to reduce Fe^{3+} of FRAP reagent to Fe^{2+}. Cytotoxic assay revealed the *P. dactylifera* showed minimum hemolysis of human RBCs as compared to *J. regia* extract. The total motility and progressive motility of human sperms were also calculated. The results showed that total motility of the sperms incubated with *P. dactylifera* extract was increased significantly ($p<0.05$) with time intervals 15, 30, 45, 60 minutes, respectively. Significant decrease in sperm motility was observed by incubating the sperms with *J. regia* extract.

Keywords: Cytotoxic, Motility, hydromethanolic, *P. dactylifera, J. regia.*

1. INTRODUCTION

Globally around 186 million world population is affected by delayed conception and inability to reproduce offsprings [1]. Many people are using advanced techniques like assisted reproductive technology (ART) to conceive the children, but these methods are very expensive and risky. It has been observed that oxidative stress plays an important role in process of conception. Oxidative stress occurs when there is an imbalance between oxidants (reactive oxygen species ROS) and antioxidants that neutralize the toxins [2]. Environmental pollution, obesity, alcohol, poor nutrition, smoking, infections and chronic diseases are the major causes that causes increases in oxidants within the body that lead to DNA damage and even apoptosis [3]. Considering the degree of

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damages that can cause by oxidative stress, it is important to find the best treatment to keep the balance between oxidants and antioxidants.

Nutraceuticals are the food items and their extracts that can provide health benefits along with nutritional benefits. They can also be used to scavenge the reactive oxygen species and prevent oxidative stress [4]. Therapeutic value of nutraceuticals includes both nutrients like carbohydrates, fats, proteins, minerals, vitamins and non-nutrients products like phytochemicals, enzyme regulators, fiber, prebiotics and probiotics [5], [6]. Plant derived items like fruits, vegetables, nuts, pulses, spices and grains have many health promoting effects due to the presence of several nutraceuticals. These nutraceuticals present in plant sources have shown different range of biological activities with lower potency as compare to synthetic drugs but their proper regular intake and controlled release may show notable long term functional effects [7] [8].

Phoenix dactylifera commonly known as date palm has a place in the *Arecaceae* family which comprises of 200 genera and 3,000 species [9]. It has been cultivated in Middle East from past 6,000 years. Date palm contains high sugar content that is around 70-80%. Majority of fructose and glucose are present in the date pulp which can be used effectively by human body [10]. They also have many kind of other important biological molecules like vitamins, fibers, fats, minerals and proteins [11]. Date fruit is a major source of antioxidants like ascorbic acid, carotenoids, polyphenols and flavonoids.

Date palm extract can be used as a remedy of multiple diseases like neural damage, liver damage, viral and bacterial infections, due to the presence of various nutraceuticals. It can also be used as an anti-inflammatory agent. Date palm extract shows many antimicrobial and antioxidant compounds which can protect against oxidative damage [12], [13].

Walnut scientifically known as *Juglans regia* belongs to the Juglandaceae family and it is the finest nuts of temperate regions [14]. Walnut is a rich source of protein and fats and its kernels mostly comprises of 70% fat and proteins present in it. Due to the presence of many antioxidants in the walnut which are more effective with respect to pure vitamin E, walnut oil and extract can be used to treat the variety of human chronic diseases [15]. Walnut has many therapeutic actives like it act as antimicrobial agent, can be used against heart diseases due to unsaturated fatty acid. Walnut possesses anti-inflammatory, antioxidants, astringent, antiseptic, anthelmintic, antidiarrheal, hypoglycemic, tonic, depurative, hypoglycemic and carminative activity [16]. Walnut oil had been used many times for the improvement of reproductive disorders by increasing the amount of testosterone [17].

The present research work is being plant to explore the nutraceutical potential of *P. dactylifera* and *J. regia* hydromethanolic extracts by exploring antioxidant potential, cytotoxic potential and *in vitro* sperm activity.

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2. METHODOLOGY

2.1. Collection and identification of food sources

Nutraceutical containing food sources *P. dactylifera* pulp and *J. regia* kernels for current study were procured from the local market of Faisalabad, Pakistan. The food items and their selected parts for nutraceutical extraction were taxonomically authenticated from Department of Botany, University of Agriculture, Faisalabad, Pakistan.

2.2. Proximate Analysis of *P. dactylifera* fruit and *J. regia* kernels

The dried powder was used to measure crude protein, carbohydrate, lipid, moisture, ash and crude fiber content by following methods of AOAC [18] in the test samples. Crude protein was estimated by multiplying the 6.25 factor with sample nitrogen content. Moisture content was obtained by oven drying the fruit samples until constant volume was obtained. Lipid content was obtained by *n*-hexane solvent extraction. Total Carbohydrates of fruits were calculated by taking difference between 100% of total nutritional value of fruit and the addition of crude protein, ash, moisture, lipid and fiber content [19].

2.3. Preparation of nutraceutical containing extracts of selected food items

The *P. dactylifera* pulp and *J. regia* kernels were washed with distilled water and dried. After drying they were converted into fine powder with the help of grinder, Model CB 222, Cambridge United Kingdom. The powdered food materials (1000 g) were extracted with methanol in water (1:5 v/v) using an orbital shaker (Gallenkamp, UK) for 72 hours at room temperature. The concentrated extracts were attained in a rotary evaporator (Heidolph, model Laborata 4000, Schwabach, Germany) under reduced pressure at 50°C. Subsequently, the extracts were put to freeze dried by applying vacuum to get rid of the remaining solvent effects [20]. The extracts, now concentrated, were weighed properly and percentage yield of nutraceutical extract (g/100 g of dry food item) was calculated by applying the following formula.

$$\text{Yield (\%)} = \frac{\text{Weight of dried nutraceutical extract}}{\text{Weight of dried food material}} \times 100$$

2.4. Antioxidant potential of nutraceutical extracts

2.4.1. Qualitative estimation of phytochemicals

Numerous phytochemical compounds alkaloids, flavonoids [21], tannins, glycosides, steroids [22] and Triterpenoids [23] present in hydromethanolic nutraceutical extract were qualified using standard protocols.

2.4.2. Total phenolic content

Folin-Ciocalteu method was used to determine total phenolic contents in methanolic food extracts. One mililitre of sample extracts was dissolved with 4 mL of 20% sodium carbonate and 5 mL of Folin-Ciocalteu reagent (diluted ten folds) [24]. The absorbance

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of blue color complex was measured after 1 hour incubation at 765 nm. Standard curve was prepared using different concentration of gallic acid which is a very good phenolic compound.

2.4.3. Total flavonoid content

Total flavonoid content of nutraceutical extracts was examined by mixing the test sample (0.5 mL) with 0.15 mL of 5% $NaNO_2$ and 2 mL of distilled water and this mixture was incubated for six minutes. After that 0.15 mL of $AlCl_3$ was added in the reaction mixture and mixture was incubated for five minutes. After the 4% NaOH solution was added to the mixture. Volume of reaction mixture was made 5 mL with the help of methanol and mixed well. Absorbance of mixture was calculated at 510 nm after 15 minutes incubation [25].

2.4.4. DPPH Inhibition Assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was used to final out the antioxidant potential of nutraceuticals as explained by Shahid *et al [26]*. One milliliter of 0.004% DPPH was prepared in methanol solution and 3 mL of food extract was added in it. Samples with DPPH were placed in dark for 30 minutes. Optical density was find out at 517 nm. Solution without sample extract was considered as blank. Percentage of DPPH radical inhibition was calculated using following formula.

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

Where; A_1 = Absorbance of extract sample, A_0 = Absorbance of blank without sample

2.4.5. Ferric reducing power assay

The nutraceutical's reducing potential was found out by reduction of Fe^{3+} $(CN)_6$ to Fe^{2+} $(CN)_6$ through direct shifting of electron [27]. Reaction mixture consists of 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1%) with 1mL nutraceutical extract. This mixture was incubated for 20 minutes in test tubes at 50°C. In each test tube stop solution 2.5 mL of TCA (1% w/v) was added. After that each mixture was centrifuged at 3000 rpm for 10 minutes. Optical density was calculated at 700 nm. Absorbance has a direct relation to the reductive potential of sample.

2.5. Cytotoxic potential of nutraceutical extracts through Hemolytic Assay

Hemolytic assay was used to determine the cytotoxic potential of extracts using human erythrocytes as described by Kumar *et al*. [28]. Percentage hemolysis of tested samples was measured spectrophotometrically by using 10 mg/mL extract mixed in 20 % DMSO solution against human RBCs. Phosphate buffer saline (PBS) was specified as negative control, while Triton X (0.1%) was considered as positive standard.

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2.6. Thrombolytic activity of nutraceutical extract by clot lysis method

Thrombolytic activity of nutraceutical extracts was carried out through *in vitro* clot lysis method as described by Tabassum *et al*. [29]. Venous blood was drawn from healthy individuals without having any history of anticoagulant therapy. Ability of test sample to dissolve the clot was compared with PBS blank and positive control streptokinase in terms of percentage of blood clot weight loss.

2.7. *In vitro* sperm motility

For *in vitro* sperm parameters healthy individuals (n=30) were selected to obtain semen samples after their consent and processed as directed by world health organization (WHO) as shown by Lu *et al*. [30] Only the normal semen samples were considered for further study to ensure the semen quality as directed by WHO reference ranges.

Physiological saline (1mL) was used to dissolve the 50µg/mL nutraceutical extract and mixed sample was kept overnight at room temperature for settlement. Sperm parameter analysis was performed by mixing equal volume of nutraceutical extract mixed with (0.9% physiological saline) and semen samples immediately.

The mixture was incubated for 30 seconds at room temperature. Sperm motility and progressive motility was measured at different time intervals of 0, 15, 30, and 60 minutes of incubation at 37°C under the light microscope as described by Khaleghi *et al*. [31].

3. RESULTS

3.1. Proximate analysis

Proximate analysis of *P. dactylifera* pulp and *J. regia* kernels is given below in table 1. The carbohydrate content of the *P. dactylifera* (45.98%) was found to be more as compared to *J. regia* (8.04%). Crude fat content of walnut kernels (67.29%) was highest as compared to *P. dactylifera* fruit. Protein content of walnut and date was found about 19.78% and 3.86% respectively.

The moisture content was found to be more in *P. dactylifera pulp* (13.81%) and it was found to be lowest in the *J. regia* kernels (2.50%). Fiber content of *P. dactylifera* was found to be more (27.01%) as compared to *J. regia* kernels.

3.2. Percentage yield

The percentage yield of *P. dactylifera* was found to be high 28.8 g / 100g of dried fruit material as compared to percentage yield of *J. regia* that was 3.96/ 100g of dried fruit material (Table 2).

The obtained methanolic extracts of nutraceutical were tested for *in vitro* biological activities like antioxidant, cytotoxic and thrombolytic activities. The effect of nutraceutical extract was evaluated on spermatozoa by spermicidal activity.

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Table 1: Nutritional composition of the selected food items

Sr. No	Parameters	*P. dactylifera*	*J. regia*
1	Crude fat %	4.25±0.18	67.29±1.99
2	Protein content %	3.86±0.05	19.78±0.21
3	Crude fiber %	27.01±0.01	5.99±0.03
4	Ash content %	5.09±0.17	1.40±0.35
5	Moisture content %	13.81±0.3	2.50±0.01
6	Carbohydrate content %	45.98	8.04

Values are Mean ± SEM (standard error of mean) of (n=3) replicate measurements.

Table 2: Extraction yields of different food items using methanol solvent

Name	Yields (%)	Color of extract
P. dactylifera	28.8	Golden brown
J. regia	3.96	Darkish brown

3.3. Qualitative and quantitative phytochemical Analysis

Methanolic extract of *P. dactylifera* shows the presence of flavonoids, glycosides and absence of saponins and tannins. Our results showed that walnut extract contains flavonoids, saponins, glycosides and alkaloids, steroids and terpenoids are absent in it (Table 3). Total phenolic and flavonoid content of selected nutraceutical extracts was evaluated in the present research work. Variations of means ± SEM of TPC and TFC are given below in the following table 4. The values have been shown as milligram of gallic acid for TPC comparable to 1 g of dry nutraceutical extracts. Our results showed highest total phenolic content of *J. regia* extract (351 ± 3.2 mg GAE/g dry food material) followed by *P. dactylifera* fruit extract (295 ± 4.2 mg GAE/g dry food material). Highest flavonoid content of *J. regia* (70.49 ± 1.54 µg CE/g dry food material) was also found followed by *P. dactylifera* fruit extract (51.63 ± 1.38 µg CE/g dry food material).

Table 3: Phytochemical screening of the studied nutraceutical extracts

Nutraceutical extracts	Alkaloids	Flavonoids	Tannins	Glycosides	Saponins	Steroids	Triterpenoids
P. dactylifera	-	+	-	+	-	+	+
J. regia	-	+	+	+	+	-	-

(+) indicates the presence of phytoconstituent, (-) indicates no phytoconstituent present

Table 4: Antioxidant potential of nutraceutical extracts through different assays

Parameters/ Sample	TPC (mg GAE/g dry food material)	TFC (µg CE/g dry food material)	Reducing power capacity	DPPH Scavenging activity (%)
P. dactylifera	295 ± 4.2^{C}	51.63 ± 1.38^{B}	1.164±0.01	79.15 ± 1.01^{C}
J. regia	351 ± 3.2^{B}	70.49 ± 1.54^{A}	1.197±0.07	80.35 ± 1.12^{C}
Vitamin C	-	-	-	93.43 ± 2.13^{A}

Note: TPC (Total Phenolic Contents), GAE (Gallic Acid Equivalent), TFC (Total Flavonoids Contents), CE (Catechin Equivalent). DPPH (1, 1-diphenyl-2-picrylhydrazyl).

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(-): No testing. All the given data for each experiment is expressed as Mean ± SEM of three replicates (n=3), Means that do not share the same alphabetic letter are significantly ($p<0.05$) different.

3.4. *In vitro* Antioxidant Profiling

The results of DPPH scavenging activity are given in the table 4 with mean ± SEM of test sample. The DPPH free radical scavenging activity of *J. regia* kernels was found to be 80.35 ± 1.12% followed by free radical scavenging activity of *P. dactylifera* pulp extract (79.15 ± 1.01%) as compared to the positive control vitamin- C DPPH scavenging activity (93.43 ± 2.13%). In the present study the methanolic extracts of selected nutraceuticals are evaluated for their reducing power ability by reducing ferric ions into ferrous ions. Variations of means ± SEM are given in table 4. Methanolic extract of *J. regia* showed the highest reducing power ability followed by *P. dactylifera* reducing ability. The increase in the reductive ability of test samples depends upon the total concentration of antioxidants that are present in the extracts.

3.5. Cytotoxic activity

Hemolytic activity of methanolic nutraceuticals extracts (10 mg/mL) was evaluated by spectrophotometric method for current studies using normal healthy blood suspensions. Hemolytic activity of study samples was expressed in percentage hemolysis and stated as mean ± standard error mean of three replicated values in figure 1. The results showed that among the methanolic extracts *P. dactylifera* showed the lowest hemolysis (7.02 ± 0.72) while on the other hand *J. regia* extract showed the highest hemolytic activity 14.56 ± 2.85. Triton X was used as a positive control and it showed the maximum hemolysis around 83.46 ± 4.75 and phosphate buffer was used as negative control and it shows the lowest hemolysis (2.33 ± 0.193) as compared to tested extracts.

3.6. Thrombolytic activity

The thrombolytic activity by % clot lysis method was used to fine the thrombolytic effect of nutraceuticals and streptokinase as positive control and distilled water as negative control. The result of thrombolytic showed that streptokinase (100 µL) after adding to blood clots causes the 75.14 ± 1.43% clot lysis after incubating for 90 min at 37ºC while negligible clot lysis (4.04 ± 0.65%) was observed after treating distilled water with clots. Among the tested nutraceutical extracts *P. dactylifera* showed the highest colt lysis percentage that was about 52.31 ± 1.61% and least activity was showed by *J. regia* (30.75 ± 1.45) (Figure 2).

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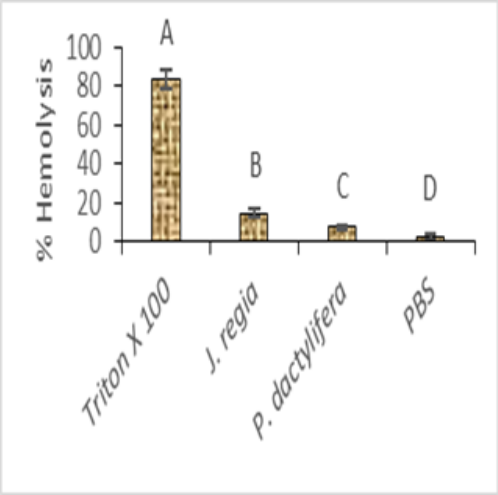

Figure 1: Percentage (%) hemolysis of washed RBCs by the studied extracts and controls.
Results are expressed as means ± SEM of control and extracts in replicates (n=3). Means that do not share the common alphabetic letter are significantly ($p<0.05$) different.

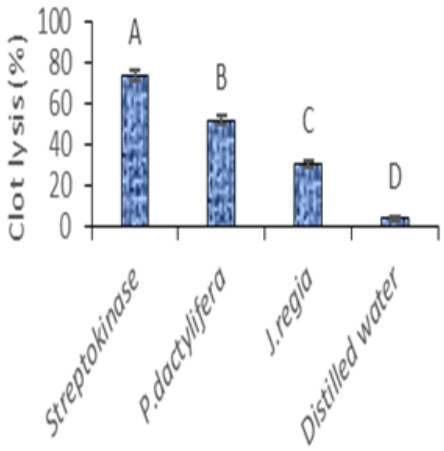

Figure 2: Clot lysis (%) of selected nutraceutical extracts.
Results are expressed as means ± SEM of control and extracts in replicates (n=3). Means that do not share the common alphabetic letter are significantly ($p<0.05$) different.

3.7. Effect of *P. dactylifera* and *J. regia* nutraceutical extracts on sperm motility

The total motility and progressive motility of human sperms was observed by incubating the sperms with control, *P. dactylifera* and *J. regia* extract respectively at different intervals of time. The results showed that total motility of the sperms incubated with *P. dactylifera* extract was increased significantly ($p<0.05$) with time intervals 15, 30, 45, 60 minutes respectively with as compared to the control (Figure 3a).

While on the other hand significant decrease in the sperm motility was seen when sperm were incubated with *J. regia* extract. Progressive motility of sperms treated with *P. dactylifera* extract was remained constant at 0 to 30 minutes time interval and it was increased significantly at 45 and 60 minutes time interval respectively shown in figure 3b.

Significant decrease in sperm progressive motility was observed by incubating the sperms with normal saline control with increased time intervals. Significant decrease in the sperm progressive motility was seen when sperm were incubated with *J. regia* extract.

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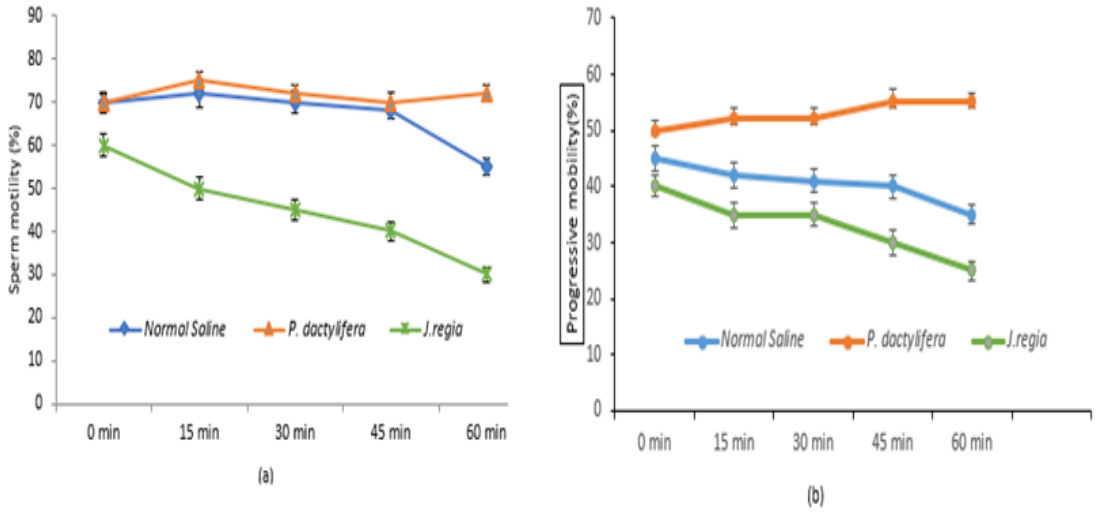

Figure 3: Effect of nutraceutical extract on sperm motility. (a) Total motility of sperms incubated with nutraceutical extracts at different time intervals. (b) Progressive motility of sperms incubated with nutraceutical extracts at different time intervals. Results are expressed as means ± SD of replicate measurements (n=3)

4. DISCUSSION

Nutritional therapy that comprises of nutraceuticals and dietary remedies is very common healing system for the body to compete against different ailments. Nutraceuticals obtained from plants and animal sources contain antioxidants that can be used as fertility boosters especially in males [32]. Nutraceutical like arginine, coenzyme Q10, carnitine, selenium, vitamin E, folic acid, zinc, vitamin A, vitamin C have important part in increasing the sperm count and motility with in limited access. But the excess of these nutraceuticals can be dangerous with mild side effects [4].

In the present research nutritional value of *P. dactylifera* and *J. regia* was observed with the help of proximate analysis. *P. dactylifera* nutraceutical extract contains high amount of sugar (Carbohydrate) that are the primary source of energy for the body [33]. The lower moisture content in the walnut kernels shows that they have longer shelf life as compared to date fruit. Jamil e*t al. [34]* also evaluated the proximate analysis for different varieties of dates and values of carbohydrate and fiber content were slightly higher from our values. The fat content of walnut kernel shows that walnut oil may contains variety of fats, omega -3 fatty acids [35]. Memon, (2019) [36] also showed the same result that walnut has highest amount of crude fat close to our value as compared to other nuts. Lower ash content of tested samples shows that they contain variety of volatile organic compounds. Proteins content shows that walnut contains significant amount of protein and dates are not a good source of protein [33].

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The qualitative estimation of phytochemicals showed the presence of flavonoids and glycosides in both tested extracts. Anjum *et al*. [37] also evaluated the various flavonoids are in date palm extract and they explained that these flavonoids belong to important class of phenolics and hence they have the ability to quench reactive oxygen species. Labyad *et al*. [38] evaluated the phytochemical detection of five different varieties of Date palms and the results shows the presence of flavonoids, phenolic compounds and absence of alkaloids and tannins as shown in our results. These flavonoids and phenolic compounds are the major antioxidants that can be used to neutralize the reactive oxygen species and treat many ailments by possessing biological activities [39]. Trandafir *et al*. [40] showed the total antioxidant activity of walnut kernels is due to the phenolic and flavonoid contents in the extract. Defects in natural anticodons system in body may cause various disorders but these food supplements like flavonoids, vitamins and carotenoids can prevent this oxidative damage [41].

DPPH scavenging method measures the direct transfer of hydrogen item or electron to scavenge the reactive free radical species. *P. dactylifera* extract showed significant scavenging activity this may be due to the presence polyphenolic compounds particularly proanthocyanidins. These are the polymers of catechin found in many dates which contributes mainly in this activity [42]. The walnut oil contains many phytochemicals that can reduce Fe^{3+} to FRAP reagent Fe^{2+} as shown in our results [43]. Three selected dates varieties were evaluated for antioxidant potential by various assays and results showed that there is a powerful connection between phenolic, flavonoid content and reducing power evaluated through FRAP test as described in our results [44].

It is important to evaluate the drug toxicity before its usage. Hemolytic activity of a therapeutic compound is a best way to find level of toxicity of that compound towards healthy blood cells. The date extract showed minimum hemolysis this may be due to presence of certain biological compounds [28]. Walnut kernel showed some hemolysis which may be due to the presence of certain oils within it. Thrombus (blood clots) formation in the circulatory system is due to the failure of the hemostasis of vascular system with in body that can leads to sever diseases. Many thrombolytic drugs have been reported by literature that can cause severe side effects like bleeding or embolism [45]. Natural fruits and plants contain many thrombolytic agents that can be a source of thrombolytic drugs from cheaper source and with mild side effects. Date extract showed significant thrombolytic activity which may be due to the presence of secondary metabolites [46].

Nutraceuticals obtained from plants and animal sources contain antioxidants that can be used as fertility boosters especially in males. Nutraceutical like arginine, coenzyme Q10, carnitine, selenium, vitamin E, folic acid, phytochemicals, zinc, vitamin A, vitamin C have important part in increasing the sperm count and motility with in limited access [47]. The sperm membrane is rich with polyunsaturated fatty acids that can be attacked easily by ROS species. These ROS species are the cause of infertility by destroying sperm structure and reducing motility through lipid peroxidation. It has been reported that

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improved sperm motility and bulkiness of sex organs directly relates to the fecundity [48], [49]. In the present research date nutraceutical extract improved the sperm parameters such as motility with different time intervals, while *J. regia* does not improved the sperm motility this may be due to the limited bioavailability of nutrients present in walnut. In a study it was observed date extract improved the sperm parameters and mobility as shown in our results [50]. Akomolafe *et al*, [51] reported that walnut extract improved the sperm parameters and mobility and their results were opposite to our study. This may be due to the selection of wrong solvent for walnut extraction.

5. CONCLUSION

The following study depicts that the *P. dactylifera* and *J. regia* nutraceutical extract contains many bioactive compounds due to which the extracts discloses the antioxidant activities, thrombolytic activities and minimal hemolysis of human RBCs. Furthermore, *P. dactylifera* hydromethanolic extract can be used to improve the sperm parameters and mobility. However, detailed study is needed to explore the particular nutraceuticals involved in whole mechanism.

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Conflict of Interest

Authors declared no conflict of interest

Ethics Approval

This research article is derived from the PhD Thesis of Miss Fatima Yousaf and the research plan was approved by the Graduate Studies of University of Agriculture, Faisalabad, Pakistan (No.DGS/32005-08 dated August 31, 2018). This study plan has been approved by the research scrutiny committee of the University of Agriculture, Faisalabad, Pakistan.

References

1) Inhorn, M.C. and P. Patrizio, *Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century.* Human reproduction update, 2015. **21**(4): p. 411-426.

2) Smits, R.M., et al., *Antioxidants in fertility: impact on male and female reproductive outcomes.* Fertility and sterility, 2018. **110**(4): p. 578-580.

3) Tremellen, K., *Oxidative stress and male infertility—a clinical perspective.* Human reproduction update, 2008. **14**(3): p. 243-258.

4) Anand, S. and N. Bharadvaja, *Potential benefits of nutraceuticals for oxidative stress management.* Revista Brasileira de Farmacognosia, 2022. **32**(2): p. 211-220.

5) Sachdeva, V., A. Roy, and N. Bharadvaja, *Current prospects of nutraceuticals: A review.* Current pharmaceutical biotechnology, 2020. **21**(10): p. 884-896.

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Vol: 67 Issue 07 | 2024
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6) Atta, A., et al., *Inhibition of oxidative stress and advanced glycation end-product formation in purified BSA/glucose glycation system by polyphenol extracts of selected nuts from Pakistan.* Food Science & Nutrition, 2023. **11**(6): p. 3414-3421.

7) Riaz, M., et al., *Phytobioactive compounds as therapeutic agents for human diseases: A review.* Food Science & Nutrition, 2023. **11**(6): p. 2500-2529.

8) Taid, T.C., R.C. Rajkhowa, and J.C. Kalita, *A study on the medicinal plants used by the local traditional healers of Dhemaji district, Assam, India for curing reproductive health related disorders.* Advances in Applied Science Research, 2014. **5**(1): p. 296-301.

9) Alhaider, I.A., et al., *Date palm (Phoenix dactylifera) fruits as a potential cardioprotective agent: The role of circulating progenitor cells.* Frontiers in pharmacology, 2017. **8**: p. 592.

10) Juhaimi, F.A., K. Ghafoor, and M.M. Özcan, *Physical and chemical properties, antioxidant activity, total phenol and mineral profile of seeds of seven different date fruit (Phoenix dactylifera L.) varieties.* International journal of food sciences and nutrition, 2012. **63**(1): p. 84-89.

11) El-Far, A., et al., *Date palm (Phoenix dactylifera): protection and remedy food.* Curr Trends Nutraceuticals, 2016. **1**(2): p. 1-10.

12) Shehzad, M., et al., *Therapeutic potential of date palm against human infertility: A review.* Metabolites, 2021. **11**(6): p. 408.

13) Nawaz, A., et al., *Antioxidant and phytochemical analysis of different date palm (Phoenix dactylifera l.) Seed varieties: An in vitro assessment.* Liaquat Medical Research Journal, 2024. **6**(1): p. 19-28.

14) Ebrahimi, S., et al., *Persian walnut composition and its importance in human health.* International Journal of Enteric Pathogens, 2018. **6**(1): p. 3-9.

15) Tapia, M.I., et al., *Comparative study of the nutritional and bioactive compounds content of four walnut (Juglans regia L.) cultivars.* Journal of Food Composition and Analysis, 2013. **31**(2): p. 232-237.

16) Mukarram, S.A., et al., *Global Perspectives on the Medicinal Implications of Green Walnut and Its Benefits: A Comprehensive Review.* Horticulturae, 2024. **10**(5): p. 433.

17) Bostani, M., et al., *A study on the effects of walnut oil on plasma levels of testosterone pre and post puberty in male rats.* American Journal of Ethnomedicine, 2014. **1**(4): p. 266-275.

18) Shahid, M., et al., *Proximate composition, antioxidant activities and fatty acid profiling of selected mushrooms collected from Azad Jammu and Kashmir.* Acta Poloniae Pharmaceutica-Drug Research, 2020. **77**(1): p. 145-153.

19) Jadid, N., et al., *Proximate composition, nutritional values and phytochemical screening of Piper retrofractum vahl. fruits.* Asian Pacific Journal of Tropical Biomedicine, 2018. **8**(1): p. 37-43.

20) Afzal, M., M. Shahid, and A. Jamil, *Phytochemical Spectrum of Essential Oil of Paganum harmala by GC-MS and Antimicrobial Activity Using Sequential Solvents Fractions and Essential Oil.* Asian Journal of Chemistry, 2014. **26**(2).

21) Jain, S., et al., *Preliminary phytochemical, pharmacognostical and physico-chemical evaluation of Cedrus deodara heartwood.* Journal of Pharmacognosy and Phytochemistry, 2014. **3**(1): p. 91-95.

22) Yadav, R. and M. Agarwala, *Phytochemical analysis of some medicinal plants.* Journal of phytology, 2011. **3**(12).

23) Ayaz, M., et al., *Phenolic contents, antioxidant and anticholinesterase potentials of crude extract, subsequent fractions and crude saponins from Polygonum hydropiper L.* BMC complementary and alternative medicine, 2014. **14**: p. 1-9.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 07 | 2024
DOI: 10.5281/zenodo.12794152

24) Liu, X., et al., *Antioxidant activities of extracts from sarcocarp of Cotoneaster multiflorus.* Journal of Chemistry, 2018. **2018**(1): p. 4619768.

25) Riaz, M., et al., *Antioxidant Profiling and Hepatoprotective Potential of Methanolic Extract of Mucuna pruriens Seeds in Male Albino Rats.* Journal of Biological Regulators and Homeostatic Agents, 2023. **37**(7).

26) Shahid Chatha, S., et al., *Bioactive components and antioxidant properties of Terminalia arjuna L.* Extracts. J Food Process Technol, 2014. **5**(298): p. 2.

27) Riaz, M., et al., *In vitro antioxidant potential of selected aphrodisiac medicinal plants.* Journal of biological regulators and homeostatic agents, 2017. **31**(2): p. 419-424.

28) Kumar, G., L. Karthik, and K.V.B. Rao, *Hemolytic activity of Indian medicinal plants towards human erythrocytes: an in vitro study.* Elixir Appl Botany, 2011. **40**(5534): p. e5537.

29) Tabassum, F., et al., *In-vitro thrombolytic activity and phytochemical evaluation of leaf extracts of four medicinal plants of Asteraceae family.* Journal of Pharmacognosy and Phytochemistry, 2017. **6**(4): p. 1166-1169.

30) Lu, J.-C., Y.-F. Huang, and N.-Q. Lü, *WHO Laboratory Manual for the Examination and Processing of Human Semen: its applicability to andrology laboratories in China.* Zhonghua nan ke xue= National journal of andrology, 2010. **16**(10): p. 867-871.

31) 31. Khaleghi, S., et al., *Tribulus terrestris extract improves human sperm parameters in vitro.* Journal of evidence-based complementary & alternative medicine, 2017. **22**(3): p. 407-412.

32) Falsig, A.M., C. Gleerup, and U. Knudsen, *The influence of omega-3 fatty acids on semen quality markers: a systematic PRISMA review.* Andrology, 2019. **7**(6): p. 794-803.

33) Agboola, O.S. and A.L. Adejumo, *Nutritional composition of the fruit of the Nigerian wild date palm, Phoenix dactylifera.* 2013.

34) Jamil, M.S., et al., *Proximate composition and mineral profile of eight different unstudied date (Phoenix dactylifera L.) varieties from Pakistan.* African Journal of Biotechnology, 2010. **9**(22): p. 3252-3259.

35) Beyhan, O., et al., *Fat, fatty acids and tocopherol content of several walnut genotypes.* Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 2017. **45**(2).

36) Memon, N.N., *Nutritional characteristics (fatty acid profile, proximate composition and dietary feature) of selected nuts available in local market.* Pakistan Journal of Analytical & Environmental Chemistry, 2019. **20**(1): p. 39-46.

37) Anjum, F.M., et al., *Phytochemical characteristics of date palm (Phoenix dactylifera) fruit extracts.* Pak J Food Sci, 2012. **22**(3): p. 117-27.

38) Labyad, N., et al., *Phytochemical screening of methanolic extract of five libyan date varieties (Phoenix dactylifera L.) and evaluation of their antimicrobial activity.* Int. J. Progress. Sci. Technol, 2020. **22**: p. 168-175.

39) Tauheeda Riaz, T.R., et al., *Enzyme inhibitory, antifungal, antibacterial and hemolytic potential of various fractions of Colebrookia oppositifolia.* 2017.

40) Trandafir, I., S. Cosmulescu, and V. Nour, *Phenolic profile and antioxidant capacity of walnut extract as influenced by the extraction method and solvent.* International Journal of Food Engineering, 2017. **13**(1): p. 20150284.

41) Jayakumar, R., et al., *Chitin scaffolds in tissue engineering.* International journal of molecular sciences, 2011. **12**(3): p. 1876-1887.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 07 | 2024
DOI: 10.5281/zenodo.12794152

42) Al-Obid, M. *Date skin as a source of antioxidant activity and free radical scavenging*. in *First International Meeting of Date Palm Phoenix dactylifera L., Qas-sim*. 2003.

43) Li, L., et al., *Polyphenolic profiles and antioxidant activities of heartnut (Juglans ailanthifolia var. cordiformis) and Persian walnut (Juglans regia L.).* Journal of agricultural and food chemistry, 2006. **54**(21): p. 8033-8040.

44) Kchaou, W., et al., *In vitro antioxidant activities of three selected dates from Tunisia (Phoenix dactylifera L.).* Journal of Chemistry, 2014. **2014**(1): p. 367681.

45) Fuentes, E., et al., *Thrombolytic/fibrinolytic mechanism of natural products.* Fibrinolysis and Thrombolysis, 2014: p. 107-121.

46) Munir, N., et al., *Phytochemical constituents and in vitro pharmacological response of Cnidium monnieri; a natural ancient medicinal herb.* Dose-Response, 2022. **20**(3): p. 15593258221115543.

47) Ko, E.Y., E.S. Sabanegh Jr, and A. Agarwal, *Male infertility testing: reactive oxygen species and antioxidant capacity.* Fertility and sterility, 2014. **102**(6): p. 1518-1527.

48) Riaz, M., et al., *Impact of reactive oxygen species on antioxidant capacity of male reproductive system.* International journal of immunopathology and pharmacology, 2016. **29**(3): p. 421-425.

49) Woode, E., A. Alhassan, and C.S. Abaidoo, *Effect of ethanolic fruit extract of Xylopia aethiopica on reproductive function of male rats.* Int J Pharm Biomed Res, 2011. **2**(3): p. 161-165.

50) Mehraban, F., et al., *Effects of date palm pollen (Phoenix dactylifera L.) and Astragalus ovinus on sperm parameters and sex hormones in adult male rats.* Iranian journal of reproductive medicine, 2014. **12**(10): p. 705.

51) Akomolafe, S.F. and G. Oboh, *Walnut leaf extract acts as a fertility agent in male Wistar albino rats–A search for herbal male fertility enhancer.* Journal of Complementary and Integrative Medicine, 2018. **15**(2).