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Immunostimulant effect of *dates (Phoenix dactylifera)* on humoral and cellular immunity cells and their functions

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Abstract

The study aimed to assess the immunomodulatory effects of *Phoenix dactylifera* (dates) fruit, a traditional remedy used by Moroccans to enhance immunity against pathogens. This research sought to evaluate the impacts of this fruit on immune cells and their functions. To achieve this, we conducted tests using date extracts on splenocytes, thymocytes, and macrophages, focusing on their functions: antibody production, phagocytosis, and T-lymphocyte toxicity. The results obtained demonstrated that the aqueous extract of *P. dactylifera* fruit exhibited significant immunostimulatory effects on humoral immunity. It achieved this by enhancing complement activity and increasing splenocyte (including B-lymphocytes) proliferation by 142.5% compared to control cells. Similarly, in the same conditions, there was notable stimulation of cellular immunity through thymocyte activity, resulting in a remarkable increase in cell proliferation (225%) and a boost in thymocyte function (245.9%), which plays a role in safeguarding against cancer. Moreover, the date extract demonstrated anti-inflammatory properties. This was evident in the increased phagocytosis activity mediated by macrophages under the ethyl acetate extract, effectively eliminating pathogens. Assessing the cosmetic potential of date extracts showed that the ethyl acetate extract possesses both anti-inflammatory and strong antioxidant effects, exhibited high photo absorption of ultraviolet-B rays. Based on these findings, we propose to study the utilization of this extract for sun protection as a sunscreen. Furthermore, the Fourier-transform infrared spectroscopy analysis indicated that the most active compounds present were flavonoids. These outcomes substantiate the traditional usage of this fruit for reinforcing immunity.

Keywords: Cell proliferation, IgG production, Immunostimulant, phagocytosis, *Phoenix dactylifera*, splenocytes, antioxidant

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Introduction

Morocco has gained renown for its rich tradition of herbal medicine. Within Moroccan culture, a plethora of applications involving pharmacological natural products exist.[1] Many of these botanical products serve to bolster the immune system's defenses against diverse microorganisms. The modulation of the immune system through various therapeutic plant products is currently undergoing extensive global investigation. Consequently, a significant proportion of contemporary medicinal drugs trace their origins back to plants utilized in traditional medicine. Multiple studies are currently underway to explore various plant species and their therapeutic potentials, with the findings garnering international attention and elevating the prominence of traditional medicine. Furthermore, according to the World Health Organization, approximately 75% of the global population primarily relies on herbal remedies for their healthcare necessities.[2,3] This underscores the keen interest in discovering novel and safe immunomodulatory agents derived from natural sources.

Phoenix dactylifera, commonly known as “Tmar” in Morocco, boasts elevated nutritional value and a composition replete with mineral salts, vitamins, organic acids, tannins, and sugars.[4] All these bioactive compounds confer advantageous effects.[5-8] In the realm of traditional medicine, Moroccans predominantly consume dates to fortify immunity against infections. Moreover, this fruit finds application in treating various ailments, notably fever, inflammation, and hypertension.[9-12] This usage is concentrated in the southern and desert regions of Morocco.

Prior studies have delved into the antioxidative and anti-inflammatory impacts of Moroccan *P. dactylifera.*[4,13] Nevertheless, exploration into other significant biological activities of this medicinal Moroccan plant remains constrained. Thus, the present inquiry delved into the immunomodulatory effects of dates on immunity, utilizing a cellular model of immune cells isolated and cultivated for assessing the fruit's immune-boosting activity. This cellular model was additionally employed to evaluate immune cell functions, particularly antibody production, T-lymphocyte toxicity, and the complement system. Additionally, we investigated the fruit's antioxidative properties, its absorption of ultraviolet-B rays, and its Fourier-transform infrared spectroscopy. This investigation employed *P. dactylifera* fruit sourced from the Daraa-Tafilalt region in the southern part of Morocco, aiming to ascertain its potential utility as a treatment against cancer and viral disorders.

Subjects and Methods

Preparation of extracts

P. dactylifera (dates) were sourced from the southern region of Morocco, specifically the Daraa-Tafilalt region. The collected dates were disinfected using 70% ethanol and then dried at 40°C until a stable weight was achieved. Subsequently, they were finely ground into a powder. The powder underwent maceration at a 10% concentration in water, ethanol (EtOH), or ethyl acetate (EtOAc). Following filtration, the water extract was lyophilized at -80°C using a freeze drier (bk-fd12p (-80), Biobase freeze dryer, Biobase Biodustry Co., Ltd (Shandong), China) for 24 hours. The ethanol and ethyl acetate extracts were evaporated using a rotary evaporator (Rotary Evaporator RE100-PRO, BIOBASE Group, Shandong, China). Finally, the extracts were preserved at -20°C until their intended use.

Cell culture

This study employed cell suspensions obtained from previously slaughtered rabbits. The rabbits, weighing between 1.5 to 2 kg, were anesthetized with petroleum ether, and then humanely slaughtered.

All chemicals were purchased from Sigma-Aldrich, USA. As delineated in our previous investigations,[14,15] the immune organs, namely the spleen and thymus, were aseptically isolated from the animals. Cell suspensions were generated by passing the organs through a fine wire mesh. Subsequently, red blood cells were lysed using 154 mM ammonium chloride after being rinsed with RPMI medium (Sigma-Aldrich- USA). Live spleen and thymic cell counts were determined microscopically using trypan blue staining, employing the 1% exclusion rate test. Spleen and thymic cells were cultured in RPMI medium supplemented with 2 mM glutamine, 10% serum, ampicillin at 100 U/mL, streptomycin at 100 mg/mL, and fluconazole at 2 mg/ml. In all these experimental series, the effect of extracts on cells were compared to a control group composed by cells alone without extracts.

Cell proliferation assay

As previously noted [16,18], cell proliferation was evaluated employing the MTT assay, following the methodology established by Mosmann et al., 1983.[19] Rabbit splenocytes and thymocytes were seeded in 96-well plates (Citotest Labware manufacturing CO., LTD, 48Xinxiu Road, Haimen City 226100 Jiangsu, China) at a density of 150,000 cells per well. Subsequently, the cells were cultured/incubated for 72 hours at 37°C in a humidified environment comprising a mixture of 95% air and 5% CO2. Prior to incubation, EtOH, EtOAc and aqueous extracts of date (*P. dactylifera*) were dissolved in RPMI at the desired concentration and introduced to the cells. Following this, 10 µL of MTT solution (5 mg/mL in PBS) was added. Following a three-hour incubation period, dimethyl sulfoxide (DMSO) was introduced to all wells to dissolve the formed formazan. Lastly, the optical density was measured at a wavelength of 570 nm using a spectrophotometer (Bio Tek L800, Bio Tek Instruments, USA).

Isolation of macrophages and evaluation of their proliferation and activity

To summarize, macrophages were isolated from spleen cells based on their adherence ability to plate wells. In this process, 100 µL of spleen cell suspension containing 2.10^6 cells/mL was added to 96-well plates, which were then incubated at 37°C for 2 hours to allow macrophages to adhere. Following this, non-adherent cells were removed, and each well was subjected to two washes using sterile RPMI.

For the assessment of macrophage proliferation, the adherent macrophages were incubated in RPMI medium containing various date extracts. The proliferation was subsequently evaluated using the MTT assay, in accordance with above-described procedures.

The phagocyte test was executed in 96-well plates following the methodology outlined in our prior studies,[14,16,17,18] utilizing neutral red. In each well, adherent macrophages were cultured in 100 µL of RPMI supplemented with 0.075% neutral red. Subsequently, plant extracts were introduced, and the plates were incubated for 2 hours at 37°C in a humidified environment comprising a mixture of 95% air and 5% CO2. To conclude, after removing the supernatant and washing the cells with RPMI, the reaction was terminated using a solution containing acetic acid (1M) and ethanol (1:1 v/v), which dissolved the phagocytosed neutral red. The extent of phagocytic activity was gauged by measuring the absorbance at 540 nm, which corresponds to the maximum absorption of neutral red employed in the assay.

Allogenic mixed lymphocyte reaction (MLR)

Freshly isolated thymocytes were incubated with Chicken Red Blood Cells (CRBC) at a ratio of 10^5 CRBCs to 10^6 thymocytes in RPMI medium supplemented with fetal bovine serum (Sigma-Aldrich, USA). The mixture was then incubated, with or without date extracts, for 24 hours at 37°C in a humidified environment. To gauge the cytotoxicity of thymocytes against CRBC, the lysis of CRBCs releases hemoglobin, the dye. The level of hemoglobin released (proportional to the number of lysed CRBC) was then measured by evaluating absorbance at 540 nm.

Complement test

The complement test was executed through the assessment of mouse red blood cell (MRBC) lysis via the complement pathway, in the presence of anti-MRBC antibodies obtained

from immunized rabbits using Freund's adjuvant and mouse RBCs as antigens. MRBCs were incubated in RPMI medium supplemented with serum containing anti-MRBC-specific antibodies for a duration of 4 hours, alongside 2 mg/ml extracts, all maintained at 37°C. Following the incubation period, the samples were centrifuged, and the absorbance of the supernatants was determined at 540 nm.

Evaluation of IgG production by an enzyme immunosorbent assay (ELISA)

The ELISA was conducted following established procedures.[14,16,17,18] Isolated splenocytes were incubated with or without date extracts at 2 mg/ml in RPMI medium for a duration of 72 hours at 37°C. Following incubation, 100 µL of cell culture supernatant was placed onto a microtiter plate for total IgG evaluation. Subsequently, peroxidase-labeled anti-IgG rabbit antibody (100 µL per well) was added and allowed to incubate for two hours at 37°C. The immune complex was then revealed by adding the chromogen orthophenylene diamine (OPD) at a concentration of 0.5 mg/ml. The reaction was halted by the addition of 3M HCl, and absorbance was measured at 490 nm.

In the case of specific IgG, the procedure commenced by coating the wells with 100 µL of Ovalbumin (at a concentration of 0.001 mg/ml). Subsequently, 100 µL of the cell culture supernatant was added, and the assay proceeded as outlined above for total IgG.

Total phenolic content

The total phenolic content of the extract was determined through the Folin-Ciocalteu method. This technique involves the meticulous mixing of 1.5 ml of Folin–Ciocalteu reagent (10%) with 200 µl of date extracts (dissolved in methanol). The resultant mixture was gently stirred and allowed to react for 5 minutes in darkness. Subsequently, 1.5 ml of sodium carbonate (5%) was introduced. After an incubation period of 2 hours at room temperature, away from light, the values were measured at 750 nm. The phenolic content's concentration was established through a calibration curve employing gallic acid as a standard solution. The outcomes were denoted in milligrams equivalent of gallic acid per gram of extract.

Total flavonoid content

The quantification of flavonoids was performed using the aluminum trichloride (AlCl3) method as described by Bahorun et al., 1996 [42]. To initiate the procedure, 0.5 ml of date extract (dissolved in methanol) was blended with 0.1 ml of aluminum chloride (10%), 0.1 ml of potassium acetate (1 M), and 4.3 ml of distilled water. Following thorough mixing and an incubation period of 30 minutes at room temperature, the absorbance was recorded at 415 nm. The flavonoid concentration was extrapolated from a calibration curve established with quercetin dehydrate (used as a standard since it is a flavonoid compound). The outcomes are presented in micrograms of quercetin equivalent per milligram of extract.

Antioxidant assays

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was conducted as previously described[4,15,20] with slight modifications. Briefly, EtOH, EtOAc and aqueous extracts of date *P. dactylifera* were diluted in methanol and then mixed with a DPPH solution. The absorbance of the resulting mixture was measured at 517 nm. The BHT (butylhydroxytoluene), vitamin E (tocopherol), and ascorbic acid (vitamin C) were used as antioxidants compounds. They were employed as positive controls.

For the Ferric Reducing Antioxidant Power (FRAP) assay, we followed the method outlined by Oyaizu, 1986.[21] EtOH, EtOAc and aqueous extracts of date *P. dactylifera* were combined with 2.5 ml of a 0.2 M phosphate buffer solution (pH 6.6) and 2.5 ml of a 1% potassium ferricyanide K3Fe (CN) 6. Following this, 10% trichloroacetic acid was added to halt the reaction. A portion (2.5 ml) of the supernatant, diluted with 2.5 ml of distilled water, was then mixed with 0.5 ml of FeCl3, and the absorbance of the resulting compound was measured at 700 nm.

The ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonate)) assay was executed as described by Re et al., 1999.[22] ABTS was dissolved in distilled water and potassium

persulfate was subsequently added. The reaction mixture was allowed to stand at room temperature in the dark for 12–16 hours. Following this, 2 ml of the diluted ABTS solution was mixed with 200 µl of each of the three extracts, allowed to sit for 1 minute, and the absorbance was measured at 734 nm.

UV Absorbance

The three date extracts were prepared at a concentration of 2 mg/ml, and their UV absorbance was examined in vitro. The absorbance values within the 290 nm to 400 nm range, corresponding to the Ultra-Violet B (UVB) and Ultra-Violet A (UVA) regions, were computed. In parallel, we assessed the UV absorption of two commercial sunscreens (CARE SUN+ SPF50 and CARE SUN+ SPF30) at 10 mg/ml under identical conditions to serve as positive controls.

Infrared spectral analysis

Infrared analysis was conducted using an infrared spectrophotometer (VERTEX 70 spectrophotometer, BRUKER, USA). A small drop of purified EtOH, EtOAc and aqueous extracts of date *P. dactylifera* were meticulously positioned onto the crystal surface and secured within the path of the infrared beam. The acquired infrared data were then cross-referenced with a table of infrared frequencies.

Statistical Analysis

Each experimental condition was replicated a minimum of three times. The presented values are expressed as means ± standard error of the mean (SEM). Statistical analysis was conducted using the analysis of variance (ANOVA) test, and statistically significant differences were recognized at $p<0.05$.

Results

Effect of P. dactylifera extracts on humoral immunity

Figure 1 illustrates the impact of *P. dactylifera* extracts obtained using ethanol (EtOH), ethyl acetate (EtOAc), and water on splenocyte proliferation. The presence of *P. dactylifera* extracts resulted in a minor inhibition of splenocyte proliferation under EtOH and EtOAc extracts (28.8% and 9.5% inhibition, respectively), while stimulation was observed with the aqueous extract at 2 mg/ml, showing a 142.5% response compared to the control cells without extracts (Figure 1).

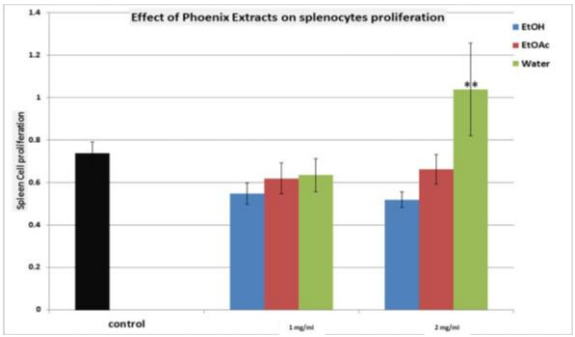


Figure 1. Effect of Phoenix extracts on splenocyte proliferation. EtOH: Ethanol extract; EtOAc: Ethyl Acetate extract; Water: Aqueous extract

Furthermore, the production of both total and specific IgG by splenocytes under different *P. dactylifera* extracts was assessed (Table 1). The aqueous extract exhibited negligible alterations in IgG production. In contrast, the EtOH and EtOAc extracts led to inhibitions of total IgG production by 25.6% and 18.8%, respectively. These inhibitory effects were more pronounced in terms of specific IgG production (EtOH: 40.1±9.2% inhibition; EtOAc: 31.3±10.3% inhibition; N=8).

Table 1. Effect of *P. dactylifera* extracts (at 2 mg/ml) on total IgG production.

Phoenix Extracts	Total IgG Production as % of Control	N
EtOH	74.4 ± 9.4 %	6
EtOAc	81.2 ± 12.5 %	6
Water	97.5 ± 6.3 %	6

Results are represented as % of control obtained in same experiments. EtOH: Ethanol extract; EtOAc: Ethyl Acetate extract; Water: Aqueous extract

Lastly, the activity of the complement system was examined under the same three extracts. Notably, the aqueous extracts significantly stimulated complement activity by 130.2%. Conversely, under identical conditions, the EtOH and EtOAc extracts induced immunosuppression of complement activity (Table 2).

Table 2. Effect of *P. dactylifera* extracts (at 2 mg/ml) on Complement activity.

Phoenix Extracts	Complement Activities as % of Control	N
EtOH	79.4 ± 13.0	6
EtOAc	83.4 ± 12.9	6
Water	130.2 ± 16.0	6

EtOH: Ethanol extract; EtOAc: Ethyl Acetate extract; Water: Aqueous extract.

Effect of P. dactylifera extracts on cellular immunity

The impact of *P. dactylifera* extracts on thymocyte proliferation was assessed. As depicted in Figure 2, the aqueous extract at a concentration of 1mg/ml exhibited a remarkable immunostimulatory effect on cell proliferation, yielding a substantial 225% response compared to control cells without extracts (p<0.001; N=5). Conversely, the EtOH extract impeded thymocyte proliferation by 28.1%, while the EtOAc extract showed no discernible effect (Figure 2).

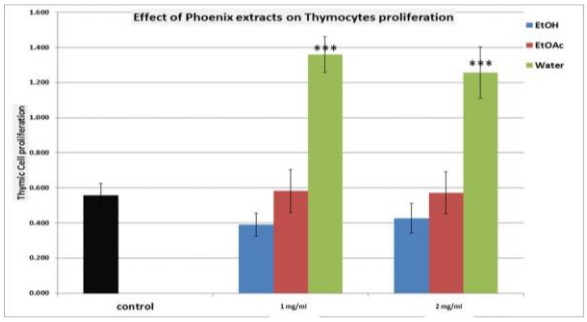


Figure 2. Effect of Phoenix extracts on thymocyte proliferation. EtOH: Ethanol extract; EtOAc: Ethyl Acetate extract; Water: Aqueous extract

Through an evaluation of thymic cell cytotoxicity against foreign cells (Table 3), it was noted that the EtOH and EtOAc extracts of *P. dactylifera* did not induce any MLR changes. Notably, however, the aqueous extract exhibited a significant augmentation in cytotoxic activity, demonstrating a prominent 245.9% response compared to the control (p<0.005).

Table 3. Effect *P. dactylifera* extracts (at 2 mg/ml) on MLR activity (Thymocyte Cytotoxicity).

Phoenix Extracts	MLR activity (Thymocyte Cytotoxicity) as % of Control	N
EtOH	93.8 ± 13.0	7
EtOAc	98.0 ± 8.5	7
Water	245.9 ± 22.0	7

Results are represented as % of the control obtained in same experiments. EtOH: Ethanol extract; EtOAc: Ethyl Acetate extract; Water: Aqueous extract.

3-Effect of *P. dactylifera* on macrophage proliferation and phagocytosis

With respect to macrophage proliferation, we observed a notable immunostimulatory effect across all extracts. This effect was particularly pronounced in the case of the EtOAc extract, displaying a peak response of 240.5% compared to the control cells without extracts (Figure 3).

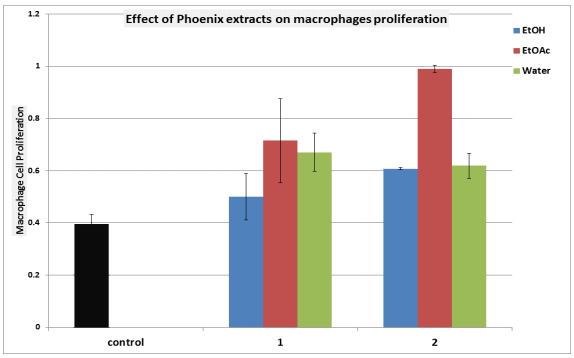


Figure 3. Effect of Phoenix extracts on macrophage proliferation. EtOH: Ethanol extract; EtOAc : Ethyl Acetate extract; Water : Aqueous extract.

Regarding phagocytosis activity (Table 4), both *P. dactylifera* EtOH and EtOAc extracts exhibited stimulating effects on phagocytosis, eliciting responses of 144% and 200%, respectively (p<0.05; N=4; Table 4). The heightened stimulation observed in macrophage proliferation and phagocytosis activities may imply an anti-inflammatory influence associated with the EtOAc extract of dates.

Table 4. Effect of *P. dactylifera* extracts on Macrophage Phagocytosis.

Phoenix Extracts	Phagocytosis of Macrophage (Absorbance)	N
Control	0.25 ± 0.01	4
EtOH	0.36 ± 0.08	4
EtOAc	0.50 ± 0.1	4
Water	0.20 ± 0.04	4

EtOH: Ethanol extract; EtOAc: Ethyl Acetate extract; Water: Aqueous extract.

Phenolic and Flavonoid content

Table 5 provides a summary of the phenolic and flavonoid content measurements obtained from *P. dactylifera* extracts. Notably, the aqueous extract exhibited a significantly elevated level of phenolic and flavonoid content, quantified at 1344.3±0.023 mg/g and 108.7±0.02 mg/g of extract, respectively.

Table 5. Total phenolic, flavonoid and antioxidant activities of *P. dactylifera* extract.

Phoenix Extracts	Total phenolic (mg of GA/g Extract)	Total flavonoid (mg of Q/g Extract)	ABTS IC50 (mg TE/edw)
EtOH	797.3± 0.01	63.7± 0.01	0.19± 0.01
EtOAc	798.8±0.01	81.2±0.01	0.19±0.001
Water	1344.3±0.02	108.7±0.02	2.66± 0.05

The values are the mean of three determinations ± standard error of the mean. EtOH: Ethanol extract; EtOAc: Ethyl Acetate extract; Water: Aqueous extract. GA: Gallic acid; Q: Quercetin; ABTS: IC50 (mg TE/edw): Trolox equivalent.

The antioxidative effect of P. dactylifera extracts

In order to assess the antioxidant activity of *P. dactylifera* extracts, three distinct methods were employed: DPPH, ABTS, and FRAP assays. Figure 4 illustrates the outcomes of the DPPH assay for all three extracts. A noteworthy antioxidant effect was observed, intensifying proportionally with concentrations ranging from 0.5 to 50 mg/ml. The EtOH and EtOAc extracts demonstrated peak antioxidant activity, exceeding 70%. The calculated half maximal inhibitory concentration (IC50) values were 2.3% for EtOAc, 0.7% for EtOH, and 4.0% for the aqueous extract (IC50 values were calculated from curve of antioxidant effect by determining the concentration needed to inhibit half of the maximum response recorded). In comparison, IC50 values were 0.55% for vitamin E, 0.06% for BHT, and 0.08% for ascorbic acid.

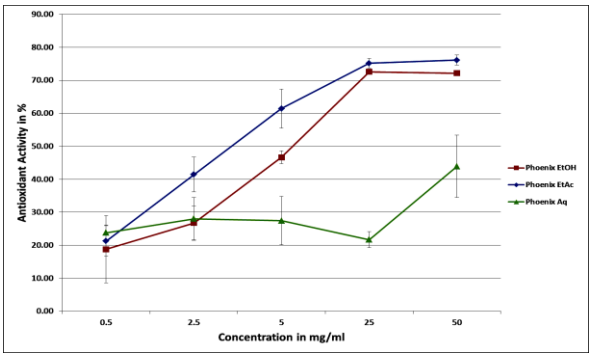
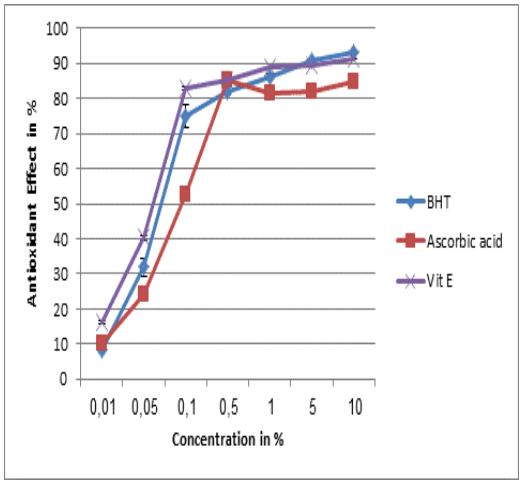


Figure 4. Antioxidative effects of *Phoenix* extracts By DPPH radical scavenging essay compared to BHT, Ascorbic acid and vitamin E. EtOH: Ethanol extract; EtOAc: Ethyl Acetate extract; Water: Aqueous extract.

These findings were corroborated by the FRAP assay, as depicted in Figure 5, reaffirming the heightened antioxidant potential of the EtOH and EtOAc extracts. Meanwhile, through the ABTS assay, we observed that the EtOH and EtOAc extracts exhibited the most potent activity, featuring an IC50 value of 0.2 g/ml.



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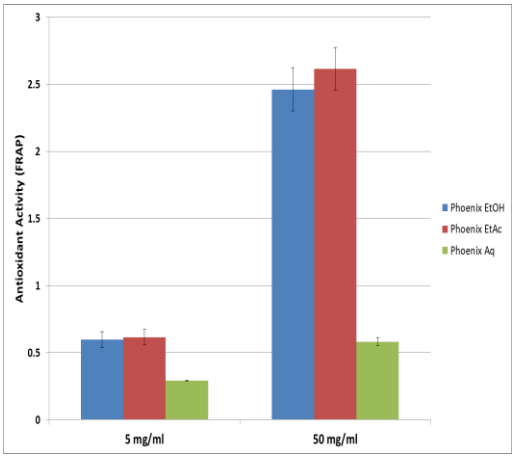

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Figure 5. Antioxidative effects of Phoenix extracts by FRAP ferric reducing antioxidant power essay compared to BHT, Ascorbic acid and vit E. EtOH: Ethanol extract; EtOAc: Ethyl Acetate extract; Water: Aqueous extract.

UV Absorbance

Figure 6 presents the UV absorbance profiles of *P. dactylifera* extracts. The findings revealed that all three extracts displayed increased absorbance for UVB as opposed to UVA. Within the UVB range, the EtOAc extract exhibited notably higher absorption compared to sunscreen Sun Protection Factor (SPF) 30, peaking at an impressive 2.5 nm. Following this, the EtOH extract registered an absorption value of 0.7 nm. Conversely, the aqueous extract demonstrated lower UVB partial absorbance, with a peak value of 0.35 nm.

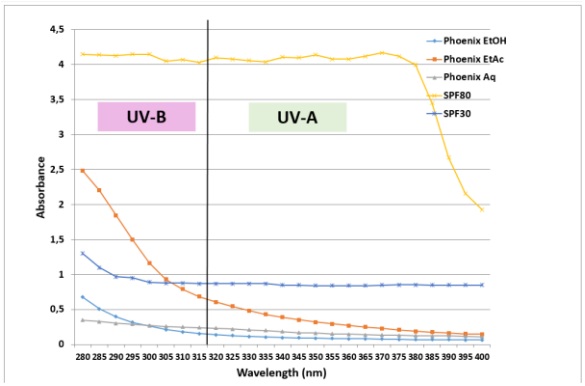

Figure 6. The UV absorption spectrum of the *Phoenix* extracts. EtOH: Ethanol extract; EtOAc: Ethyl Acetate extract; Water: Aqueous extract.

Infrared spectral analysis

To discern the underlying functional groups within the structures, FT-IR spectroscopy was employed. The infrared spectra of *P. dactylifera* extracts are presented in Figure 7. A broad band, centered around 3350 cm^{-1}, was ascribed to stretching vibrations of O−H or phenol groups.[27] A faint band between 2800-3000 cm^{-1} was allocated to symmetrical stretching vibrations of C-H[28] and/or the stretching vibrations of C-H within aromatic frameworks such as flavonoids or aromatic acids.[29] The observed band ranging from 1650 to 1750 cm^{-1} corresponds to the elongation vibration of carbonyl (C=O) bonds in flavonoids or polyphenols, along with the C=C stretching associated with aromatics. Further distinct peaks related to the stretching of C-C and the bending vibration of C-H within aromatic rings were evident around 1450 cm^{-1}, while the C-O group of hydroxy-flavonoids was detected around 1250 cm^{-1}.

The bands positioned between 1450-1200 cm^{-1}, indicative of asymmetric CH2/CH3 scissoring, and those between 500 and 850 cm^{1}, representing C-H vibrations (bending, rocking, wagging), were more pronounced in the EtOAc extract in comparison to EtOH and aqueous extracts. This heightened presence, owing to their non-polar nature, potentially affirms the presence of triglycerides or phospholipids within the extracts.[27,29] (Table 6)

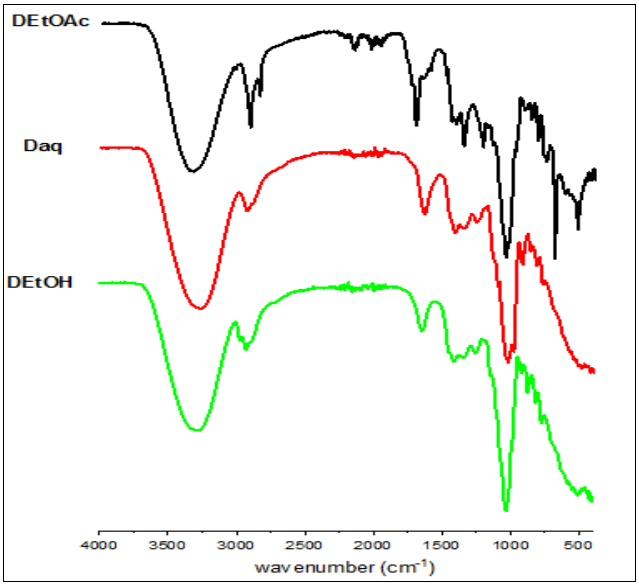

Figure 7. Fourier Transform infrared spectroscopy spectrum analysis of *P. dactylifera* extracts. DEtOH: Ethanol extract; DEtOAc: Ethyl Acetate extract; Water: Aqueous extract.

Table 6. Infrared band assignments of *Phoenix dactylifera* samples extracted with the three following solvents: EtOH, EtOAc, and water.

Wavelength number (cm^{-1})	D (EtOH)	D (EtOAc)	D (Water)	Band assignments	Reference
3640-3050 centered at 3320	3640-3050 centered at 3320	3640-3050 centered at 3320	3080-3000 centered at 3321	νO-H of alcohol and phenol groups strongly associated with *inter*- and *intra*-molecular hydrogen-bond (*broad* and *intense*)	(36) (39)
3030	-.	+	-.	νC=H_{ar} and/or νC=H of double band	
2980	+	-.	-.	$\nu_s CH_2 + \nu_{as} CH_3$ of aliphatic and/or alkyl chains	(37)
2922	+	+	+		
2865	+	+	+	$\nu_s CH_3$	(38)
2150	-.		-.	$\nu_s CH_2$	
2050	-.	+	-.	νC≡N	
2000 to 1850	-.	+.	+.	νC≡C harmonic (overtone) of the aromatic ring (*weak*)	(39)
1740	-.	+	+		
1711	-.	+	+		
1650	+	-.	+	νC=O of normal ester group (-CO_2R)	(37)
1632	+	+	-.	νC=O of ketonic group	
1600 and 1590	+	+	-.	νC=O of diconjugate carbonyl	(40)
	+	+	-.	δOH of adsorbed water (*broad*)	(38)
1465	+	+	+	$\nu C_{ar}=C_{ar}$ (stretching mode of aromatic ring)	
	+	+	-.		
1430				$\nu_r=C_{ar}$	(37)
1406	-.	-.	+	$\delta_{as} CH_2$	
1358	-.	+	-.		
1329	+	-.	+	δCH_2 (*scissoring*)	
	+	-.	+	δO-H phenolic (*broad*)	(36)

Table 6. Continued.

Wavelength number (cm^{-1})	D (EtOH)	D (EtOAc)	D (Water)	Band assignments	Reference
1246	-.	+	-.		(41)
1220	+	+	+		
1160	-.	-.	+		(36)
1120	-.	+	+		
1060 to 1040	+	-.	-.	$\delta_s CH_3$ δCH_2 (*wagging*)	(41)
998	-.	-.	+		
981	+	+	+	vC_{ar}-O guaiacyl+ vC-O of carbohydrates	(39,41)
916	+	-.	-.	vC_{ar}-O syringyl+ vC-O-C of ester (C(=O)-O-C)	(39)
881	-.	+	+	v_{as}C-O-C: bridge of β-(1-4)-glycosidic	
862	+	-.	+		
820	-.	+	-.	vC-O of secondary alcohol of carbohydrates	
790	-.	+	-.		
785	-.	+	+	vC-O of glucopyranoside ring (*broad* and *intense*): fingerprint region of carbohydrates	
779	+	-.	-.		
770	-.	+	-.		
696	-.	+	-.	…..	
650	-.	+	-.		
528	+	-.	-.	…….	
511	+	+	+		
450-900				δ_{oop}C-H of aromatic ring γC-H_{ar} (2C-H_{ar} adjacent) out of plane bending γC-H_{ar} of 1,2,4-trisubstituted aromatic δ_{oop}OH of alcohols and phenolic groups (large band)	

v: stretching vibration, v_{as}: asymmetric stretching vibration. n, v_s: symmetric stretching vibration, δ: in plane bending vibration, γ: out of plane bending mode, δ_{oop}: out of plane bending vibration, r: rocking.

Discussion

This study aimed to assess the immunity-related characteristics of a significant food source both consumed by Moroccans and globally, namely *P. dactylifera*, or dates. We scrutinized humoral and cellular immunity cells, along with their activities within a cell culture model. Three extracts were derived from dates utilizing Ethanol, Ethyl acetate, and water.

The results obtained unveil that the aqueous extract exhibited pronounced stimulation in splenocyte proliferation, composing an average of 45% B-lymphocytes, 35% T-lymphocytes, and 15% phagocytic cells the composition of spleen cells B, T lymphocytes and macrophages in percentage according to Aggarwal et al., 2013,[23] while not inducing stimulation of IgG secreted by B-lymphocytes. Similarly, under the same conditions, we observed elevated stimulation in thymocyte proliferation and their mixed cytotoxicity function (MLR). These findings imply that the aqueous extract holds potential as an immunostimulatory, particularly for

cellular immunity, which is pivotal in safeguarding against viral disorders and cancer. This conclusion finds support in two previous studies demonstrated that dates fostered natural killer cell proliferation, thus halting the progression of breast cancer.[24,25]

In contrast, in the present study, both Ethanol and Ethyl acetate extracts exhibited an immunosuppressive effect on both humoral (evidenced through inhibition of splenocyte proliferation and IgG production) and cellular immunity (as seen in the inhibition of thymocyte proliferation). This suggests that compounds extracted through these solvents could be harnessed for their potential to dampen immune reactions, serving as anti-inflammatory agents. This deduction gains strength from observations on phagocytic cells and their function, as both extracts stimulated macrophage proliferation and phagocytosis, corroborating their anti-inflammatory actions. Notably, the effect was more pronounced with the Ethyl acetate extract, suggesting its potential as a source of anti-inflammatory compounds, a notion supported by a previous study, which indicated the stimulation of phagocytic activity by *P. dactylifera.*[26]

Moreover, methanolic, acidic, and basic ethanolic extracts of date fruit (at 1 µg/mL) reduced inflammation in RAW macrophages cell-line induced by *Escherichia coli* lipopolysaccharide (LPS). The anti-inflammatory traits of dates were attributed to the presence of flavonoids and phenolic substances, as highlighted by Das et al., 2015.[27] This aligns with another research which suggested that both *P. dactylifera* extracts, laden with polyphenols (797.29±0.006; 798.79±0.012 mg of GA/g Extract) and flavonoids (63.66±0.006; 81.16±0.006 mg of Q/g Extract), possess anti-inflammatory actions.[28]

To summarize, the aqueous extract exhibited cellular immunity immuno-stimulation, while Ethanol and Ethyl acetate extracts demonstrated immunosuppression. This inhibition indicates that these extracts blocked the cell proliferation and consequently immunity reactions induced by the three immunity cell populations. This leads to suppression of inflammatory reactions, which are a part of immunity reactions. This indicates an immunosuppressive activity and therefore anti-inflammatory properties.

Lastly, a novel exploration into photo absorption along with antioxidative attributes was undertaken. The results revealed that the aqueous extract demonstrated limited antioxidant impact without significant UV ray absorption. Conversely, other extracts showcased heightened antioxidative potency, as corroborated by three distinct methods (DPPH, FRAP, and ABTS assays), thereby validating earlier findings of other studies.[4,7,29,30,31,32] Notably, the Ethyl acetate extract from palm dates demonstrated substantial photo absorption, particularly within the UV-B range.

In light of these results, the Ethyl acetate extract presents a potential application as a sunscreen due to its possession of three key attributes: (i) substantial UV-B ray absorption (>50% of UV-B rays), (ii) antioxidative characteristics, and (iii) anti-inflammatory effects. These combined properties suggest possible use of this extract in sunscreens as a skin protector by complementing these in vitro studies with in vivo studies.

By infrared spectral analysis, we observed the presence of flavonoids or aromatic acids, which were reported by another study.[33] Quercetin, isoquercetin, luteolin, rutin, apigenin are the probable flavonoids present in *P. dactylifera.*[33,34] Moreover, polyphenols was presented with higher content.[33] We also observed the presence of triglycerides or phospholipids that is present with low level in date fruit [34,35]

Through the assessment of various extracts derived from palm dates fruit, several conclusions can be drawn: (i) The aqueous extract of *P. dactylifera* fruit exhibited a more pronounced immunostimulatory impact on cellular immunity than on humoral immunity. (ii) Ethanol and Ethyl acetate extracts displayed immunosuppressive activities, indicating their potential for anti-inflammatory applications. (iii) Notably, the Ethyl acetate extract demonstrated a photoprotective action against UV-B rays, accompanied by a potent antioxidative effect, thereby suggesting its potential use as a skin protector, however, this needs further studies.

Author Contributions

LA and SL conceived the original idea and were in charge of overall direction and planning. SL, NA, and SJ carried out the experiments. IO contributed to statistical analysis. BA contributed to the chemical characterization. SL, LA, and IO wrote the manuscript with support from IO, NA, and AB.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Local Ethical Committee of Sidi Mohamed Ben Abdellah University under reference number 13 /2021/CEFST. All studies were carried out according to guidelines of animal care as prescribed by national ethical standards.

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