Nor. Afr. J. Food Nutr. Res. 2024; Vol. 8, No. 18, 253–261

https://doi.org/10.51745/najfnr.8.18.253-261

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ORIGINAL ARTICLE

Food Chemistry, Engineering, Processing and Packaging Nutritional Immunology and Reproduction

Immune-stimulating and antioxidant properties of a traditional Algerian plant combination: Date fruit (*Phoenix dactylifera*) and Fenugreek seeds (*Trigonella foenum-graecum*)

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ABSTRACT

Background: Combinatory therapy involving medicinal plants utilizes blends of different species to enhance therapeutic efficacy, increase potency, and minimize adverse effects. This approach leverages the synergistic interactions of plant-derived bioactive compounds, offering a natural and holistic strategy for addressing various health conditions.

Aims: This study aimed to evaluate the effects of a traditional Algerian combination of date fruit (*Phoenix dactylifera*) and fenugreek seeds (*Trigonella foenum-graecum*) on phagocytic activity and hepatic glutathione (GSH) levels.

Materials and Methods: Phagocytic activity was measured using the carbon clearance rate test, while hepatic glutathione levels were determined spectrophotometrically from the liver homogenates.

Results: The results demonstrated that both phagocytic activity and GSH levels were significantly enhanced in animals treated with the plant combination ($p < 0.001$). The carbon clearance rate was significantly faster in mice receiving the combined treatment (FG "Fenugreek" / PD "*Phoenix dactylifera*": 14.66 ± 4.143) compared to NaCl control group (49.77 ± 16.98). Additionally, the phagocytic index was significantly higher in the FG / PD group (7.128 ± 0.823) compared to groups treated with either fenugreek alone (FG: 4.082 ± 0.306) or date fruit alone (PD: 4.87 ± 0.608). Hepatic GSH levels were also significantly elevated in the FG / PD group compared to the other groups ($p = 0.040$).

Conclusions: The combination of date fruit and fenugreek seeds exhibits a synergic effect, enhancing immune system activity and antioxidant capacity. These findings suggest its potential for the development of novel, naturally derived therapeutic agents.

Keywords: Phagocytic activity, date fruit, fenugreek seeds, GSH, combination therapy.

ARTICLE INFORMATION

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Received: July 08, 2024
Revised: October 09, 2024
Accepted: December 03, 2024
Published: December 05, 2024

Article edited by:
Prof. Khaled Méghit Boumédiène
Article reviewed by:
Dr. Nadjet Debbache-Benaida
Dr. Lila Boulekbache-Makhlouf

Cite this article as: Kehili, H.E., Zerizer, S., (2024). Immune-stimulating and antioxidant properties of a traditional Algerian plant combination: Date fruit (Phoenix dactylifera) and Fenugreek seeds (Trigonella foenum-graecum). *The North African Journal of Food and Nutrition Research, 8* (18): 253–261. https://doi.org/10.51745/najfnr.8.18.253-261

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1 Introduction

Throughout history, humanity has encountered a wide array of illnesses and maladies, prompting the exploration of various therapeutic strategies to combat them. Among these, the use of medicinal plants has been a cornerstone of traditional medicine. Despite significant advancement in modern therapeutic approaches, the tendency toward herbal medicine continues to rise, driven largely by concerns over the toxicity associated with several first-line synthetic treatments. In recent years, the integration of medicinal plants with other therapies has been increasingly documented as a complementary and alternative treatment strategy (Nwozo *et al.*, 2023; Salmerón-Manzano *et al.*, 2020).

Medicinal plants serve as a primary source of numerous pharmacological agents, offering therapeutic solutions for a broad spectrum of illnesses. Since ancient times, natural products derived from plants have been widely utilized for both the treatment and prevention of diseases. Their continued popularity is attributed to their perceived efficacy, affordability, and reduced side effects compared to synthetic pharmaceuticals (Alkahtani *et al.*, 2022). The preparation of traditional herbal remedies often involves combination therapies, a practice rooted in the origins of therapeutic practice. Combination therapy has demonstrated significant benefits, especially for patients suffering from severe infections caused by drug-resistant pathogens (Donkor *et al.*, 2023).

A notable application of medicinal plants lies in their immunomodulatory properties. Immunomodulators are defined by their capacity to modify or regulate specific immune functions, presenting a promising alternative to conventional therapies for various diseases (Alanazi *et al.*, 2023). The bidirectional regulation of immune stimulation and immunosuppression, is vital for maintaining immune homeostasis. Immune stimulation, specifically, involves the positive modulation of the immune system to enhance the body's immune response, promote the secretion of immune factors, and facilitate the elimination of antigens. Recent advancements have highlighted the potential of natural protein modules in regulating immune functions. These modules stimulate the generation of effector cells and immune molecules (Zhang *et al.*, 2023).

Antioxidants play a crucial role in protecting cells from damage caused by free radicals. These compounds have the capacity to terminate chain reactions and inhibit oxidation processes by oxidizing themselves, and neutralizing radical intermediates. The body employs a range of endogenous and exogenous antioxidants to mitigate the formation of free radicals or limit their detrimental effects. Endogenous antioxidants include enzymatic compounds such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH). Exogenous antioxidants, on the other hand, are derived from dietary sources, including vitamins A, C, and E, minerals, and plant-derived polyphenols (Nwozo *et al.*, 2023). Among the endogenous antioxidants, the glutathione-dependent defense system is particularly noteworthy. Glutathione (GSH), a tripeptide composed of glycine, cysteine, and glutamic acid, is considered one of the most critical antioxidants due to its multifaceted role in maintaining intracellular redox homeostasis, promoting growth and repair, and supporting tissue function (Golubkova *et al.*, 2023). Reduced glutathione (GSH) is the most abundant non-protein thiol compound synthesized within the cytosol and is pivotal in balancing oxidative and antioxidative processes. During reactive oxygen species (ROS) scavenging, GSH is oxidized to form glutathione disulfide (GSSG) (Xiong *et al.*, 2021).

The date palm (*Phoenix dactylifera*), is one of the oldest cultivated fruit crops, renowned for its nutritional and medicinal attributes. Predominantly grown in tropical and subtropical regions, especially in the Middle East and North Africa, its global production has seen significant growth, with an estimated production value exceeding $14 billion in 2020 (Alkhoori *et al.*, 2022). Algeria recognized as the world's third- largest producer of dates, cultivates over 900,000 tons annually across approximately 950 varieties. Notable Algerian cultivars include *Delget Nour*, *Degla Beida,* and *Mech Degla* (Chergui *et al.*, 2021).

Date fruits are extensively utilized in traditional medicine for their therapeutic potential, to treat cancer, a number of infectious, and immunomodulatory disorders. Therefore, it has been discovered that frequent date consumption provides some protection against malignancies of the colon, prostate, breast, endometrial, lung, and pancreas. (Essa *et al.*, 2016). Botanically classified as a monocotyledonous angiosperm belonging to the Arecaceae family, the species name of *Phoenix dactylifera* reflects its unique characteristics, with “phoenix” signifying from Greek the fruit's purple or red hues and “dactylifera” translating to "finger-bearing" a nod to its fruit clusters' appearance (Oluyele *et al.*, 2022).

Dates are distinguished by their exceptional nutritional and functional properties. They are rich in simple carbohydrates, primarily glucose, fructose, alongside a host of secondary metabolites contributing to their health benefits (Al-Mssallem *et al.*, 2020).

Fenugreek (*Trigonella foenum-graecum* L.) is a plant native to Asia with over 32 identified species. Fenugreek is recognized as one of the oldest cultivated medicinal herbs in history. Widely cultivated in southern Europe, northern and eastern Africa, the name *Trigonella* originates from Latin, meaning “little triangle”, referencing the plant's triangular flowers. The ancient Greek physician Hippocrates regarded fenugreek as a relaxing herb (Sun *et al.*, 2021).

The seeds of fenugreek are aromatic, possessing a sweet or slightly bitter flavor. The seeds are rich polysaccharide, such as galactomannans, and exhibit a range of biological activities including antidiabetic, anti-inflammatory, antimicrobial, and hypocholesterolemic effects. Fenugreek seeds are also an excellent source of proteins, dietary fibers, crude fats, and essential minerals. Due to its ideal nutritional composition and functional properties, fenugreek and its bioactive components are increasingly utilized in pharmaceutical and nutraceutical applications as health-promoting foods (Dhull *et al.*, 2022).

In Algeria, the combination of dates and fenugreek has been traditionally used for various applications. This mixture is particularly favored by women postpartum for its perceived benefits in stimulating milk production, treating anemia and hair loss, and accelerating the healing of fractured bones.

The aim of this study was to evaluate the immunomodulatory and antioxidant effects of this traditional Algerian combination of date fruit (*Phoenix dactylifera*) and fenugreek seeds (*Trigonella foenum-graecum*) through both *in vivo* and *in vitro* experiments. Specifically, the study focused on the impact of the combination on on phagocytic activity within the reticuloendothelial system (RES) and hepatic glutathione levels. By investigating these effects, the research seeks to identify potential nutritional interventions to enhance population health and prevent diet-related diseases.

2 Materials and Methods

2.1 Plant material

The date jam, locally known as "Roub," was prepared using date fruits (*Phoenix dactylifera*) of the *Degla* variety, harvested at the ripening stage from Tolga, Biskra, Algeria. The Fenugreek seeds (*Trigonella foenum-graecum* L.) were purchased from the local spice market of Constantine, Algeria. The seeds were ground using a mortar and sifted to obtain a fine powder suitable for further use.

2.2 Traditional preparations of date jam ("*Roub*")

The preparation of *Roub* followed a traditional Algerian recipe. Initially, 3000 g of dates were cleaned thoroughly with water. A deep pot halfway with water (double the volume of the dates), was heated to a medium temperature (50 to 70 °C) and boiled for approximately 1.5 hours. After heat treatment, the dates were mashed, and the seeds were removed to create a homogenous purée. This purée was then pressed through a strainer to extract the juice. The juice underwent a second heat treatment (approximately 70 °C) to evaporate the water content, reducing it to around 1 Kg of a thick, syrup-like liquid. The resulting *Roub* was allowed to cool and subsequently stored in a clean jar, preserved in a warm, dark environment.

2.3 Traditional preparation of the combination

The *Roub* was combined with the powdered Fenugreek seeds according to the traditional Algerian preparation method, using a ratio of 75% *Roub* and 25% fenugreek powder. For experimental purposes, 50 mg of the fenugreek seed powder *Roub*, and the combination of both were each separately diluted in 10 mL of 0.9% NaCl solution.

2.4 Animals

Adult male albino mice (*Mus musculus*), aged 2 - 3 months and weighing 20 – 30 g, were procured from the Central Pharmacy in Algeria. The animals were housed under standard laboratory conditions, including ambient room temperature and a 12-hour light/dark cycle, in polyacrylic cages. The mice were provided with dry pellet food and had unlimited access to water. A one-week acclimatization period was observed before the initiation of the experiments.

2.5 Ethics statement

The animal experimentation was conducted in accordance with the procedures outlined in the research project (code number: F00920140076) approved by the Algerian Ministry of Higher Education and Scientific Research. All experimental procedures adhered strictly to the ethical principles and guidelines established by the Organization for Economic Co-operation and Development (OECD) for the care, monitoring, and supervision of animal in research (OECD Test No. 420, 2002).

2.6 Acute toxicity

A limit dose of 2000 mg/kg of fenugreek seed powder, date *Roub,* and their combination was administered (separately) to five healthy male adult mice. The animals were fasted overnight, with only water provided, before dosing. Each mouse was weighed prior to the oral administration of the substances.

A single mouse received a 2000 mg/kg oral dose and was monitored for mortality and clinical signs, including unusual aggressiveness, vocalization, restlessness, sedation, and somnolence. Observations were made during the first hour, every hour for the subsequent three hours, and periodically up to 48 hours. If the first mouse survived, four additional mice were sequentially dosed with the same 2000 mg/kg at 48 – hour intervals. All mice were observed closely for a period of 14 days, and any mortalities during the experiment were recorded. The median lethal dose (LD_{50}) was predicted to be greater than 2000 mg/kg if three or more mice survived the test (Ezeh *et al.*, 2021).

2.7 Phagocytic activity

The phagocytic activity of reticuloendothelial systems (RES) was assessed using the carbon clearance test. The phagocytic index, representing the rate of carbon elimination by the RES, was determined following the method described by Dillasamola *et al.* (2018).

Mice were randomly divided into four groups, each consisting of seven animals:

- **Group I (Control):** Administered 0.5 mL of 0.9% NaCl.
- **Group II:** Administered 50 mg/kg of fenugreek seed powder (FG).
- **Group III:** Administered 50 mg/kg of date *Roub* (PD).
- **Group IV:** Administered 50 mg/kg of the combination (FG/PD).

The treatments were delivered via intraperitoneal (i.p.) injection.

Forty-eight hours post-injection, a carbon ink suspension (prepared by mixing 3 mL of black carbon ink, 4 mL of saline, and 4 ml of 3 % gelatin solution) was injected into the tail vein at a dose of 0.1 mL/10 g body weight. Blood samples (approximately 25 µL) were collected from the retro-orbital plexus at 5 minutes (T1), and 15 minutes (T2) after carbon injection, using heparinized glass capillaries. The blood samples were lysed in 4 mL of 0.1 % sodium carbonate solution, and the optical density of the lysate was measured spectrophotometrically at 676 nm.

- **Phagocytic Index (K):** Reflects the total activity of the RES in contact with the bloodstream.
- **Corrected Phagocytic Index (α):** Measures RES activity per unit weight of active organs (liver and spleen).
- **Carbon Clearance Half-life ($T_{1/2}$):** Represents the time required for the carbon concentration in the blood to decrease by half.

These parameters are calculated using the following formulas:

$$K = \frac{LOG\ OD1\ - LOG\ OD2}{T2\ - T1} \qquad T\,{}^{1}\!/_{2} = \frac{0.693}{K}$$

$$\alpha = \sqrt[3]{K}\ \frac{Body\ weight\ of\ the\ animal}{liver\ weight\ + spleen\ weight}$$

Where:

- K: Phagocytic Index
- α: Corrected Phagocytic Index
- ($T_{1/2}$): Carbon Clearance Half-life

OD1 and OD2 are the optical densities at time T1 and T2, respectively.

2.8 Antioxidant activity

The antioxidant activity was measured through the spectrophotometric quantification of glutathione (GSH) in liver homogenate using 5,5-dithiobis-(2 nitrobenzoic acid) (DTNB) as the chromogenic reagent (Vuolo *et al.*, 2022).

At the conclusion of the immunostimulatory experiment, the animals were sacrificed, and their organs (liver and spleen) were carefully dissected and immediately weighed in their wet state. Liver homogenates were subsequently prepared and utilized for GSH quantification.

2.9 Statistical analysis

Statistical analyses were conducted to evaluate differences among groups subjected to various dietary treatments using a one–way ANOVA followed by Tukey's multiple comparison tests (IBM SPSS Statistics, version 20). Significance levels were set at $p < 0.05$, $p < 0.01$, and $p < 0.001$, with lower values indicating statistical significance.

3 Results

3.1 Acute oral toxicity

The results presented in Table 1 indicate that an oral dose of 2000 mg/kg did not produce any visible signs of toxicity or result in mortality. Five male adult mice were administered the same extract at this dose and monitored over a 14-day period. All mice survived without any adverse effects throughout the study duration.

Table 1. Toxic effects of date *Roub*, Fenugreek seeds powder and the combination of both plants

Limit dose			Signs of toxicity for 14 days			Signs of mortality for 14 days			Number of mice survived for 14 days		
PD	FG	FG / PD	PD	FG	FG / PD	PD	FG	FG / PD	PD	FG	FG/PD
	2000 mg/kg			No signs			No signs			5 mice	

Note. PD: *Phoenix dactylifera*; FG: Fenugreek

3.2 Evaluation of the immunomodulatory and antioxidant activities

3.2.1 Phagocytic index K

The phagocytic index (K) exhibited significant differences among the treatment groups (NaCl, FG, PD, and FG/PD), with $p < 0.001$ (Figure 1). Furthermore, the results showed that the phagocytic index values of the treated groups (FG group = 0.0168 ± 0.0017, $p > 0.05$; PD group = 0.023 ± 0.0037, $p > 0.05$) were higher than that of the control group (NaCl = 0.0151 ± 0.0046). The combination group (FG/PD) demonstrated the highest phagocytic index (0.052 ± 0.018) $p < 0.001$.

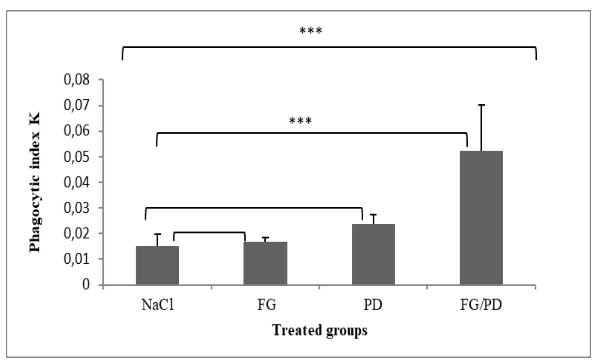

Figure 1. Effect on the phagocytic activity (K)

*Results are shown as mean ± SD (n=7) and significant difference from the control group is shown as *p < 0.05, **p < 0.01, ***p < 0.001*

3.2.2 Half-time carbon clearance from blood (T½)

The data presented in Figure 2 reveal a highly significant difference in the half-time (T½) of carbon clearance from the blood among the groups (NaCl, FG, PD, and FG/PD), with $p < 0.001$. The mean carbon clearance rates indicate that the clearance process was significantly faster in the FG/PD group (14.66 ± 4.14 min) compared to the NaCl group (49.77 ± 16.98, $p < 0.001$) and the other treated groups (FG = 43.37 ± 5.58 min, $p = 0.55$; PD = 30.00 ± 4.53 min, $p = 0.022$).

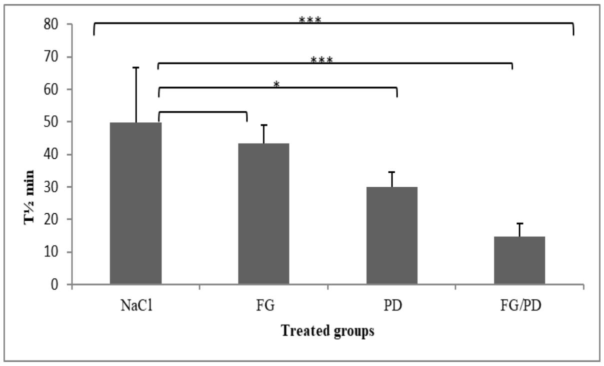

Figure 2. Effect on the half-time of carbon clearance from the blood ($T_{½}$)
*Results are shown as mean ± SD (n=7) and significant difference from the control group is shown as *p < 0.05, **p < 0.01, ***p < 0.001*

3.2.3 Corrected phagocytic index (α)

The results displayed in Figure 3 show a highly significant difference in the corrected phagocytic index (α) across the groups (NaCl, FG, PD, and FG/PD), with $p < 0.001$. The FG/PD group exhibited the highest corrected phagocytic index (7.128 ± 0.82), which was significantly greater than the values recorded for the other treated groups (PD = 4.875 ± 0.60, $p = 0.026$; FG = 4.082 ± 0.30, $p = 0.69$) and the control group (NaCl = 3.664 ± 0.112, $p < 0.001$).

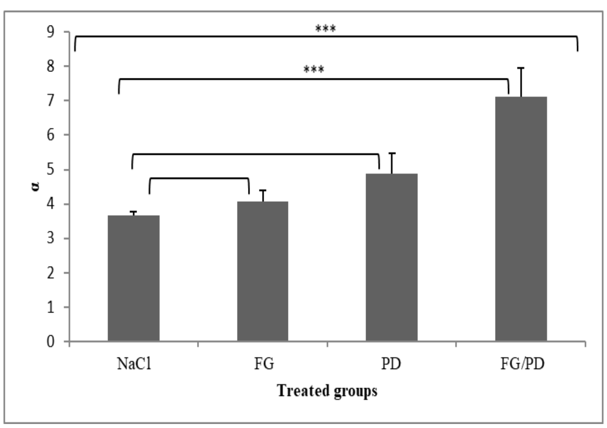

Figure 3. Effect on the corrected phagocytic index (α)
*Results are shown as mean ± SD (n=7) and significant difference from the control group is shown as *p < 0.05, **p < 0.01, ***p < 0.001*

3.2.4 Glutathione GSH

The findings illustrated in Figure 4 indicate a significant variation in glutathione (GSH) levels among the groups ($p = 0.040$). The FG/PD group demonstrated the highest GSH value (0.0083 nmol ± 0.001), which was significantly greater than the control group (NaCl = 0.000924 nmol ± 0.02, $p = 0.033$) and the other treated groups (PD = 0.0050 nmol ± 0.73, $p > 0.05$; FG = 0.0028 nmol ± 0.013, $p > 0.05$).

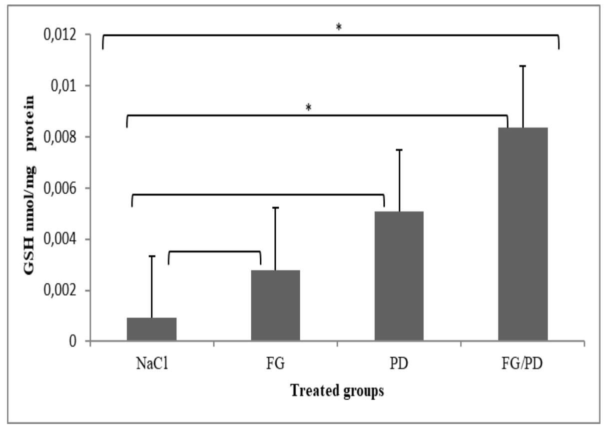

Figure 4. Effect on the Glutathione (GSH)
*Results are shown as mean ± SD (n=7) and significant difference from the control group is shown as *p < 0.05, **p < 0.01, ***p < 0.001*

4 Discussion

The results of this study revealed a significant enhancement in phagocytic activity and hepatic GSH levels in groups treated with fenugreek seeds and date *Roub* independently. These findings align with those of Osman *et al.* (2020), who reported that *P. dactylifera* exhibits substantial potential as an adjuvant in immune modulation, emphasizing its critical role in humoral immunity and its capacity to augment immune responses *in vivo*. Similarly, Oriade *et al.* (2021) highlighted the ability of *P. dactylifera* to enhance immune responses, improve phagocytic activity, and increase phagocytic index. Moreover, the study conducted by Bouhlali *et al.* (2021) indicated that aqueous extracts of date seeds significantly enhanced both enzymatic (e.g., superoxide dismutase [SOD] and catalase [CAT]) and non-enzymatic (e.g., GSH) antioxidant levels. Abdelghffar *et al.* (2022) further corroborated the antioxidant potential of *P. dactylifera*, as a natural antioxidant fruit and demonstrated that *Ajwa* dates (*Phoenix dactylifera* L.) reduce oxidative stress in cisplatin-induced nephrotoxicity by downregulating NADPH oxidase-4 (Nox4) expression. Their findings also highlighted the inactivation of reactive species such as hydrogen peroxide

(H_2O_2), nitric oxide (NO), myeloperoxidase (MPO), monocyte chemotactic protein-1 (MCP-1), along with the stimulation of antioxidant molecules, including GSH, SOD, and CAT.

The results of the current study are also consistent with those of Almatroodi *et al.* (2021), and Visuvanathan *et al.* (2022) who emphasized the immunomodulatory and antioxidant properties of fenugreek (*Trigonella foenum-graecum*). Additionally, Sharma *et al.* (2020) reported the protective effect of *Trigonella foenum-graecum* against diabetes-induced oxidative DNA damage in rats. Their findings demonstrated a reduction in superoxide anion levels, coupled with a simultaneous increase in total antioxidant capacity as well as improved ratios of non-enzymatic antioxidants, such as reduced and oxidized glutathione (GSH/GSSG) and reduced and oxidized pyridine nucleotides (NAD(P)H/NAD(P)).

The observed stimulation of the phagocytic system cells, including macrophages and neutrophils, underscores the immune response's capacity to combat invading antigens through the release of proteins such as lysozyme, peroxidases, elastases, and reactive oxygen species (ROS) like superoxide, hydrogen peroxide, hypohalous acid, and hydroxyl radicals. Excessive ROS production, however, is implicated in the pathogenesis of numerous diseases due to their potential to damage physiologically significant molecules. The prevailing understanding is that antioxidants scavenge the harmful effects of ROS by neutralizing them, thereby offering protection against oxidative stress-related diseases (Haenen *et al.*, 2014). Antioxidants are molecules that inhibit the oxidation of other substances, effectively safeguarding biomolecules from the deleterious effects of ROS. Consequently, several phytochemicals derived from vegetables and fruits have been extensively utilized for their robust antioxidant potential (Pisoschi *et al.*, 2021).

Medicinal plants play a pivotal role in preventing diseases caused by various pathogenic microorganisms by modulating the immune system, thereby functioning as potent immunomodulatory agents. Plant-based Alkaloids like berberine and piperine, terpenoids like oleanolic acid and ursolic acid, and phenolic compounds like curcumin and luteolin have demonstrated immunostimulatory activities. It has also been demonstrated that high molecular weight substances such as lectins and polysaccharides (such as pectin and glucan) might alter immune responses (Nikiema *et al.*, 2024). In addition, these organic molecules, products of secondary metabolism, exhibit a variety of biological functions, with anti-inflammatory and antioxidant properties being particularly notable (Nunes *et al.*, 2020).

The date fruit is a nutrient-dense food which may explain its biological activities such as protein, lipids, fatty acids, water, minerals, and vitamins, and provides an excellent source of energy because of its high carbohydrate content (Bentrad *et al.*, 2020). Additionally, dates are abundant in bioactive compounds such as phenolic compounds (e.g., catechin, vanillin, luteolin, tyrosol, and oleuropein), flavonoids (e.g., protocatechuic acid, caffeic acid, catechin, epicatechin, rutin, daidzein, chrysin, gallic acid, syringic acid, *p*-coumaric acid, ferulic acid, and *t*-cinnamic acid), flavonols, and condensed tannins (Bendiab *et al.*, 2021; Harkat *et al.*, 2022; Messaoudi *et al.*, 2021).

Fenugreek (*Trigonella foenum-graecum*), one of the oldest medicinal herbs, has long been valued for its therapeutic properties, with both seeds and leaves utilized for treating a range of ailments. Fenugreek seeds are a rich source of bioactive compounds, including carbohydrates, proteins high in tryptophan and lysine, fixed oils, pyridine alkaloids (e.g., choline, trigonelline, gentianine, and carpaine), flavonoids (e.g., apigenin, orientin, luteolin, quercetin, vitexin, and isovitexin), and free amino acids (e.g., 4-hydroxyisoleucine, arginine, lysine, and histidine). Additionally, fenugreek contains calcium and iron, saponins, cholesterol and sitosterol, and nicotinic acid, and volatile oils (n-alkanes and sesquiterpenes), phosphorus, riboflavin, carotene, thiamine, niacin, ascorbic acid, and β carotene. (Dhull *et al.*, 2022; Nalbantova *et al.*, 2023; Visuvanathan *et al.*, 2022; Yao *et al.*, 2020).

The findings of this study reveal a significant enhancement in phagocytic and antioxidant activities not only in groups treated with fenugreek seeds or date *Roub* separately but, more importantly, in the group treated with the combination of the two plants. This superior effect can be attributed to a synergistic interaction between the bioactive compounds of both plants. These results are consistent with the findings of Zahiruddin *et al.* (2022) who reported that a combination of extracts from four plants (*Phyllanthus emblica* L., *Piper nigrum* L., *Withania somnifera* L., and *Tinospora cordifolia)* exhibited potent immunomodulatory activity by stimulating pinocytosis and splenocyte proliferation in cyclophosphamide-induced immunosuppressed mice. This combination also enhanced the subsets of various innate and adaptive immune cells, including natural killer (NK) cells, B cells, CD4, and CD8 T cells.

Similarly, Guo *et al.* (2023) reported that a combination of Galla Chinensis, Mangosteen Shell extracts as well as the effective parts of Pomegranate peel and Scutellaria baicalensis Georgi extracts, significantly enhance immune-related enzyme activity. Furthermore, Choe *et al.* (2022) reported the antioxidant efficacy of a polyherbal combination containing eight herbal extracts (*Alnus japonica, Pinus densiflora,* and *Eleutherococcus senticosus, Camellia sinensis, Pueraria lobate, Panax ginseng* Berry, *Hovenia dulcis* Fruit, and *Opuntia humifusa*). This formulation enhanced the antioxidant defense system by elevating GSH and SOD levels.

Limitations of the study

This study demonstrates significant effects on phagocytic activity and hepatic glutathione levels; however, it does not delve into the specific molecular mechanisms or pathways through which the combination of date fruit and fenugreek exerts these effects. Furthermore, the results are derived from animal models, requiring further research to determine their applicability to human physiology.

5 Conclusions

In summary, this study provides compelling evidence that the combination of date fruit (*Phoenix dactylifera*) and fenugreek seeds (*Trigonella foenum-graecum*) exerts a synergistic effect on both immunomodulatory and antioxidant activities. This synergy is reflected in the enhanced functionality of the reticuloendothelial system, manifested by increased phagocytic activity, and elevated glutathione (GSH) levels, a critical component in maintaining cellular antioxidant defense. The remarkable potential of this traditional herbal combination can be attributed to the diverse array of phytochemicals and bioactive compounds inherent in both date fruit and fenugreek seeds, including polyphenols, flavonoids, and saponins, widely recognized for their immunostimulatory and antioxidative properties.

Despite these promising findings, the study underscores the necessity for further investigation. Future research should focus on identifying and isolating the specific bioactive components responsible for these immunostimulatory and antioxidant effects. Elucidating the precise mechanisms and interactions of these compounds will not only enhance our knowledge of the therapeutic potential of this herbal combination but also pave the way for its clinical application as a natural remedy to boosting immune function and oxidative stress defense.

Acknowledgment: The authors are grateful to the MESRS (Ministry of Scientific Research, Algeria).

Source of funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Previous submissions: The manuscript was not presented previously.

Authors' Contribution: In this study both Kehili and Zerizer were responsible for the experimental part for the biological activities and the reduction of the manuscript. **Kehili:** Conceptualization, methodology, data curation, formal analysis, software, writing - original draft, project administration. **Zerizer:** investigation, visualization, review & editing, project administration, validation.

Conflicts of Interest: No potential conflict of interest was reported by the authors.

Preprint deposit: Authors did not share this manuscript as a preprint deposit.

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