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Exploring Antioxidant Activity of Date and *Fenugreek* Essential Oils: Investigating Individual and Combined Effects Using *In vitro* and *In Silico* Analysis

ABSTRACT

Antioxidants play a pivotal role in mitigating the damaging effects of oxidative stress, which is a major contributor to the development of various diseases. *Fenugreek* (*Trigonella foenum-graecum*), known for its wide range of therapeutic properties, contains bioactive compounds that have been shown to counteract oxidative stress. Similarly, dates (*Phoenix dactylifera*) are widely used for their nutritional value and health-promoting benefits, particularly in their capacity to protect against chronic diseases. Although the individual antioxidant properties of *fenugreek* and dates are well-established, their combined effects have yet to be comprehensively explored. This study seeks to address this gap by investigating the antioxidant potential of a *fenugreek* oil and date oil combination. To test our hypothesis, we employed *in vitro* and *in silico* methods. Specifically, laboratory experiments were conducted to assess the radical scavenging activity of *fenugreek* oil, date oil, and their combination (mixture) using the DPPH assay. Concurrently, the antioxidant potential of the mixture was evaluated through *in silico* analysis using the PASS (Prediction of Activity Spectra for Substances) computational tool. Our findings revealed that the combination of *fenugreek* and date palm oils exhibited a dose-dependent increase in antioxidant activity. In addition, the *in silico* results suggested that *fenugreek* and date oils exhibit comparable antioxidant activity, with date oil displaying a slightly higher range. Consequently, we hypothesize that the combined oils would retain a similar antioxidant effect, although possibly reduced somewhat compared to their individual effects. In conclusion, *fenugreek* and date palm oils independently demonstrate significant antioxidant properties. However, these oils exhibit a marginally reduced overall antioxidant effect when combined, providing new insights into their effects following *in vitro* analysis.

KEYWORDS: Antioxidant, Date oil *(Phoenix dactylifera)*, *Fenugreek* oil *(Trigonella foenum-graecum)*, *In vitro* methods, *In silico* analysis

INTRODUCTION

Antioxidants play a key role in protecting human bodies against the harmful effects of oxidative stress, a significant contributor to various diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions (Aziz et al., 2019).

Fenugreek (*Trigonella foenum-graecum*) and date (*Phoenix dactylifera*), revered for centuries for their culinary and medicinal significance, are now emerging as potent antioxidative stress foods, showcasing their ability to combat free radicals and protect cells from oxidative damage, adding them to the list of promising natural defense sources that combat oxidative damage (Akbari et al., 2019; Al-Shwyeh, 2019). This recent discovery emphasizes the importance of utilizing known and safe edible natural antioxidant stores to improve health and well-being (Almatroodi et al., 2021).

Fenugreek is a versatile herb with a rich history dating back thousands of years. It has been esteemed for its medicinal and therapeutic properties across diverse cultures. Scientific exploration has revealed *fenugreek*'s remarkable ability to combat oxidative stress, a process implicated in numerous chronic diseases ranging from diabetes to cardiovascular disorders. Its antioxidant activity is attributed to many bioactive compounds, including flavonoids, alkaloids, and saponins, which synergize the eradication of the harmful effects of free radicals and mitigate cellular damage (Akbari et al., 2019; Almatroodi et al., 2021)

Dates, the succulent fruits harvested from the date palm tree (*Phoenix dactylifera*), have long been celebrated for their exquisite taste and remarkable health-promoting properties (Alahyane et al., 2019). Revered as a symbol of vitality and longevity in many cultures, dates have earned their place as a nutritional powerhouse enriched with antioxidants. Studies have elucidated significant antioxidant activity in dates, attributable to their diverse bioactive compounds, including phenolic compounds, flavonoids, carotenoids, and tocopherols, exerting a potent scavenging effect against free radicals (Metoui et al., 2018). Furthermore, research suggests that the consumption of dates may confer protection against chronic diseases due to their ability to combat inflammation and oxidative damage at the cellular level (Metoui et al., 2018; Hussain et al., 2020).

The chemical composition varies between the date and *fenugreek* oils. For *fenugreek,* the chemical composition responsible for its antioxidant activity includes phenolic compounds, flavonoids, alkaloids, and saponins. Phenolic compounds, such as coumarins and flavanols, exhibit strong antioxidant properties, scavenging free radicals that prevent cellular oxidative damage. Flavonoids, like quercetin and rutin, contribute to *fenugreek*'s antioxidant capacity by neutralizing reactive oxygen species. Alkaloids, including trigonelline, have been shown to possess antioxidant effects, aiding in the protection against oxidative stress-related ailments. In addition, saponins found in *fenugreek* exhibit antioxidant activity, further enhancing its health-promoting properties. Together, these chemical constituents make fenugreek a valuable natural source of antioxidants, supporting overall well-being and potentially mitigating various chronic diseases (Niknam et al., 2021; Benziane et al., 2019; Kumar et al., 2021).

On the other hand, the antioxidant activity of date palms (*Phoenix dactylifera*) is primarily attributed to various chemical groups, including phenolic compounds, flavonoids, carotenoids, and tocopherols. Phenolic compounds, such as caffeic acid and ferulic acid, are known for their potent antioxidant properties, helping to neutralize free radicals and prevent cellular damage. Flavonoids, such as quercetin and kaempferol, contribute further to the antioxidant capacity of dates by scavenging reactive oxygen species. Carotenoids, like β-carotene and lutein, give dates their vibrant color and possess antioxidant properties that protect against oxidative stress. Additionally, tocopherols, a form of vitamin E, play a vital role in combating free radicals and maintaining cellular health. Together, these chemical groups make dates a potent source of natural antioxidants, promoting overall well-being and potentially reducing the risk of chronic diseases (Metoui et al., 2018; Mohamed et al., 2021; Hussain et al., 2019; Ourradi et al., 2021). Previous studies have

individually demonstrated the antioxidant properties of date and *fenugreek* oils. However, there is a lack of research exploring their combined antioxidant activity and understanding the mechanisms underlying any potential effects of their combination. This gap underscores the need for comprehensive investigations into the antioxidant properties of herbal combinations (Al-Shwyeh, 2019; Singh, 2023).

The proposed study addresses this gap by comparing both *in vitro* (lab experiments) and *in silico* study data for each oil and their combined effects. This approach will provide a holistic understanding of the antioxidant activity of date and *fenugreek* oils, thereby contributing to the advancement of research and development in the field of antioxidants.

MATERIALS AND METHODS

The study investigates the antioxidant impact of date and *fenugreek* oils separately and in combination via *in silico* and *in vitro* analysis. A DPPH (2,2-diphenyl-1-picrylhydrazyl) assay performed to assess antioxidant activity in vitro, while the PASS online web server was utilized to evaluate antioxidant activity *in silico*, as shown in **Figure 1.**

Sample Collection

The essential oil samples were procured via the online platform of Hemani Herbal, Pakistan, a reputable company specializing in producing and distributing essential oils (https://pk.hemaniherbals.com/). Freshly obtained samples of *fenugreek* essential oil (*Trigonella foenum-graecum L.*) and date oil (*Phoenix dactylifera L.*) were subjected to comprehensive *in vitro* and *in silico* analyses to assess their antioxidant potential and associated bioactive properties.

Preparation of Oil Samples

The oil samples, including fenugreek oil, date oil, and their combination, were mixed with a DPPH solution (reagent) and 99% ethanol (solvent) in test tubes at various oil concentrations (analyte) (20 µl, 40 µl, 60 µl, 80 µl, and 100 µl) as illustrated in **Table 1**.

Table 1: The oil samples' concentration, including *fenugreek* and date oils, and their combination.

Oil type	*Concentration*
Fenugreek oil	0 *µl*, 20 *µl*,40 *µl*,60 *µl*, 80 *µl*, and 100 *µl*
Date oil	0 *µl*, 20 *µl*,40 *µl*,60 *µl*, 80 *µl*, and 100 *µl*
Combination of both oils (1:1)*	0 *µl*, 20 *µl*,40 *µl*,60 *µl*, 80 *µl*, and 100 *µl*

* *Note: (1:1) ratio was used in the combination samples because both date and fenugreek oils had almost similar densities. Fenugreek oil = 0.8051g/ml, Date oil = 0.8526g/ml* (Noureddini et al., 2016).

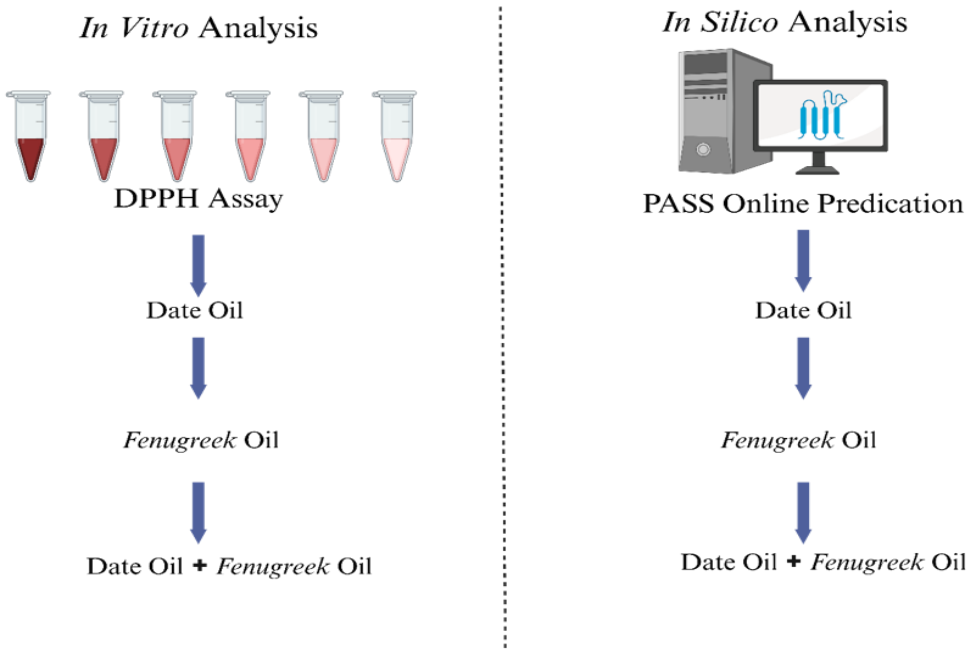

Figure 1. Schematic representation of the study workflow, combining the DPPH assay (left) to assess antioxidant activity and in silico analysis (right) to predict molecular interactions, providing comprehensive insights into the oils' antioxidant properties.

The total volume of each sample was 4 ml, comprising 2.4 ml of DPPH solution and 1.6 ml of ethanol. Notably, the volume of ethanol was adjusted proportionally with the increase in the sample concentration to ensure a consistent total volume of 4 ml in each sample. The samples were vigorously shaken for 10 minutes in each test tube to ensure proper interaction between the oil components and DPPH. Then, the mixtures were allowed to stand for an additional 10 minutes in the dark to facilitate the reaction between the antioxidants in the oil and the DPPH radical. The samples were centrifuged for 5 minutes to separate the water and oil phases. The supernatant layer was carefully collected for further analysis, leaving behind the oil phase. This step helps remove oil components that may cause turbidity and interference during absorbance measurement.

In Vitro Analysis via Determination of DPPH Measuring Radical Scavenging Activity

The baseline correction (control) uses a blank solution comprising 1.6 ml of ethanol solvent and 2.4 ml of DPPH solution. To ensure precise and reliable data acquisition, the absorbance measurements were recorded at a wavelength of 517 nm using a UV-1900i spectrophotometer. The antioxidant activity of the oil samples is evaluated by quantifying the reduction in absorbance compared to the blank solution, employing established computational methods to obtain accurate results. Additionally, each step in the experiment was performed in triplicate to ensure consistency in the results.

Measurement of Absorbance at 517nm

Absorbance was measured at 517 nm for all 18 samples (comprising six distinct samples, each analyzed in triplicate). This precise wavelength is selected due to its alignment with the peak absorption of the DPPH radical, thereby ensuring optimal sensitivity and accuracy in detecting radical concentration. The continuous

monitoring of absorbance at 517 nm enables the quantitative evaluation of the samples' capacity to neutralize DPPH radicals, serving as a robust and reliable measure of their antioxidant activity under controlled experimental conditions.

Antioxidant Activity Calculation

The absorbance was measured at a wavelength of 517 nm, and the antioxidant activity, expressed as the percentage of DPPH radical scavenging activity, was determined using the following equation:

$$DPPH\ Radical\ Scavenging\ Activity\ (\%) = \frac{(A_{control} - A_{sample}) \times 100}{A_{control}}$$

In this formula, $A_{control}$ represents the absorbance of the DPPH solution in ethanol without the oil sample. A_{sample} corresponds to the absorbance of the mixture containing the oil sample at various concentrations, including ethanol and DPPH solution. The reaction mechanism involves the interaction of the DPPH radical with antioxidant compounds capable of donating hydrogen atoms, leading to the reduction of DPPH as represented by the equation:

$$DPPH^* + RH \rightarrow DPPH_2 + R^*$$

This reduction is accompanied by a colorimetric change from deep violet to light yellow, indicative of the neutralization of DPPH radicals. The degree of this color change, a quantitative measure of antioxidant activity, was recorded at 517 nm using a UV-visible spectrophotometer.

Data Analysis

The absorbance values for each sample and concentration were recorded using a UV-visible spectrophotometer. Subsequently, the antioxidant activity of each sample concentration was calculated using the formula for DPPH radical scavenging activity shown in section 2.3.2.

Statistical analysis was performed to evaluate the antioxidant efficacy of fenugreek oil, date oil, and their combinations at various concentrations. The analysis included determining the half-maximal inhibitory concentration (IC_{50}) value, representing the concentration required to inhibit 50% DPPH radicals. Thus, the IC50 value provides a quantitative measure of antioxidant potency.

Determination of IC_{50}

The IC_{50} was determined through graphical analysis. A scatter plot was constructed, with percentage inhibition plotted on the y-axis and sample concentrations (mg/mL) on the x-axis, reflecting a linear relationship. The IC_{50} value was calculated using the equation of the simple linear regression line *(y=mx+c)*, where y represents percentage inhibition, x denotes the sample concentration, and m and c are the slope and intercept, respectively.

In Silico Prediction Using PASS Online

The antioxidant potential of the mixture was assessed using the computer-based tool PASS (Prediction of Activity Spectra for Substances) (http://www.pharmaexpert.ru/passonline/). The software utilizes structure-activity relationships to forecast the biological actions of chemical structures, including phytochemicals, by

comparing them to a known chemical entity (Jamkhande et al., 2016). The system forecasts the desired pharmacological outcome and the molecular mechanisms of action and undesired side effects such as mutagenicity, carcinogenicity, teratogenicity, and embryotoxicity. The system compares the structure with a training set comprising over 205,000 chemicals, which display more than 3750 different types of biological activity. The activity is quantified using P_a (probable activity) and P_i (probable inactivity). Only substances with a P_a value greater than P_i were examined for a specific pharmacological activity. Additionally, computational simulations were employed to model and analyze the properties of *fenugreek*, date, and their combined oil samples. Moderately active (scoring between 0.50 and 0.00) or inactive (scoring less than 0.50) against the examined targets. (https://www.molinspiration.com/cgi-bin/properties) (Kulkarni et al., 2022).

RESULTS

In Vitro Analysis:

IC_{50} values serve as crucial indicators of potency, elucidating the concentration at which oils exert their inhibitory effect on DPPH radicals. A potent effect is interpreted as having lower IC_{50} values, indicating higher antioxidant activity. The antioxidant activities of *fenugreek* oil, date palm oil, and their combination were evaluated using the DPPH scavenging assay.

Investigating the antioxidant activities of *fenugreek* oil, date palm oil, and their combination, **Table 2** presents the inhibition percentage of DPPH radicals at various concentrations for each oil, with samples 1 to 5 representing increasing concentrations.

Table 2: Percentage inhibition of DPPH radicals by *fenugreek* oil, date palm oil, and their combination at various concentrations.

	***Fenugreek* Oil**		**Date Palm Oil**		***Fenugreek* and Date Palm Oils Combination**	
Sample	Conc (mg/ml)	Inhibition (%)	Conc (mg/ml)	Inhibition (%)	Conc (mg/ml)	Inhibition (%)
1	4.03	21.41802	4.20	24.29629	4.43	11.68224
2	8.06	45.64254	8.40	56.74074	8.86	24.61059
3	12.09	59.82275	12.60	60.59259	13.30	48.28660
4	16.12	82.27474	16.81	69.48148	17.73	57.00935
5	20.15	83.30871	21.01	85.92592	22.16	88.00623

Fenugreek oil demonstrated a dose-dependent increase in antioxidant activity, reaching 83.31% inhibition at 20.15 mg/ml. Similarly, date palm oil exhibited a dose-dependent increase in antioxidant activity, with 85.93% inhibition at 21.01 mg/ml. The combination of fenugreek and date palm oils reached 88% at 22.16 mg/ml, showing a dose-dependent increase in antioxidant activity, as depicted in **Table 2**. This correlation highlights the trend of increasing antioxidant activity with higher oil concentrations.

The antioxidant activities of *fenugreek* oil, date palm oil, and their combination were evaluated using the DPPH scavenging assay, and the IC_{50} values are illustrated in **Figure 2**.

Fenugreek oil exhibited notable antioxidant activity with an IC_{50} value of 9.9513 mg/ml. This potency can be attributed to its composition, particularly palmitic acid and phytol, as detected by GC-MS analysis (Akbari et al., 2019). The observed IC_{50} value indicates a significant ability of *fenugreek* oil to scavenge free radicals, potentially offering protective effects against oxidative stress. Similarly, date palm oil exhibited a potent antioxidant activity with an IC_{50} value of 9.70 mg/ml, highlighting its rich antioxidant content, likely including compounds such as gallic, protocatechuic, p-coumaric, and ferulic acid (Harkat et al., 2022).

These antioxidants are crucial in neutralizing free radicals and mitigating oxidative damage. Date palm oil's effectiveness in the DPPH scavenging assay suggests its potential utility as a natural antioxidant source for various applications. Surprisingly, the combination of *fenugreek* and date palm oils resulted in an IC50 value of 14.28 mg/ml, indicating a lower antioxidant activity than the individual oils. This unexpected outcome suggests the combination may not have yielded a synergistic effect in this assay. The lack of synergistic effect could be due to various factors, such as the specific ratios of the oils in combination or potential interactions between their components. Further investigations are required to understand the mechanisms underlying the interaction of these oils and their impact on antioxidant activity. These findings raise intriguing questions regarding the optimal blending ratios for synergistic antioxidant effects.

In Silico Analysis:

Molecular Target Predictions Using Molinspiration:

Molinspiration Cheminformatics tools* were used to predict the molecular targets for the identified metabolites, which provide an estimated bioactivity score for metabolites against a variety of biological targets such as G protein-coupled receptors (GPCR), ion channels, nuclear receptors, kinases, Protein-disulfide reductase (glutathione) inhibitor, proteases, and enzymes. The bioactivity score value categorizes substances as active (if the anticipated score equals or exceeds 0.00), *fenugreek* Oil Identified compounds. The chemical structures of the previously identified active ingredients from *fenugreek* and date oils are shown in **Figures 3** and **4**. Additionally, the percentages of the active ingredients from the *fenugreek* and date oils are shown in **Tables 3** and **4**.

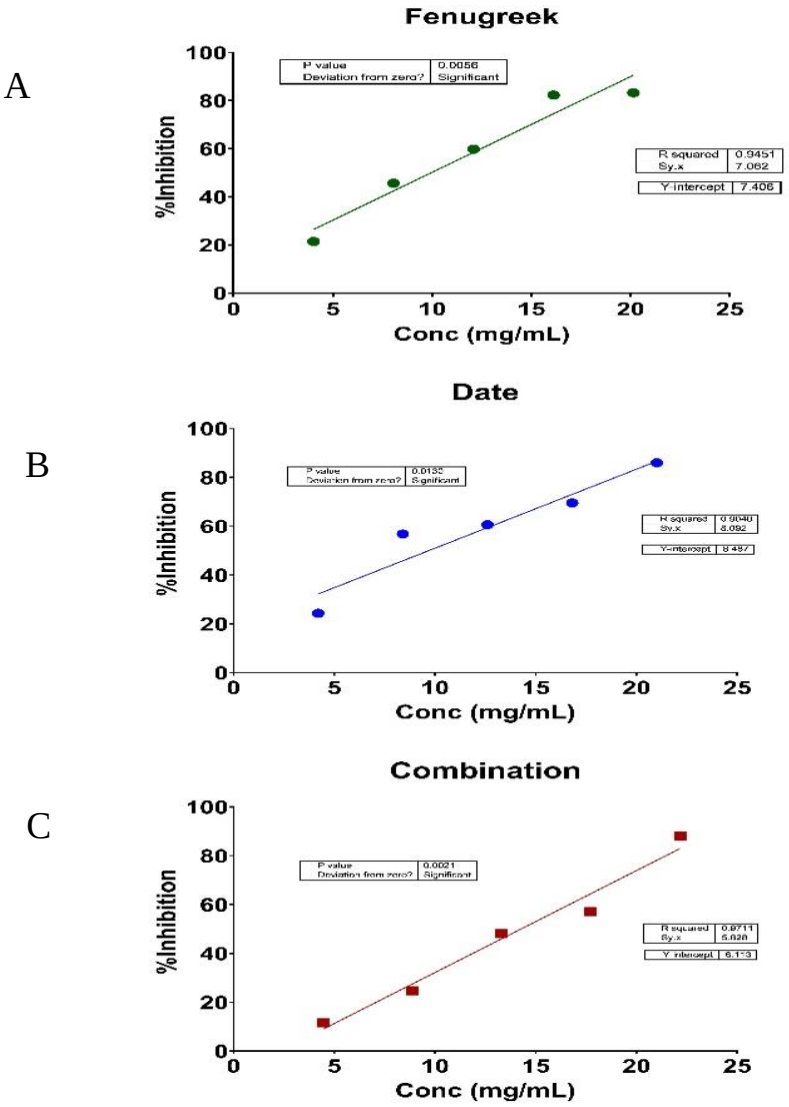

Figure 2: A, B, and C illustrate the correlation between concentration and inhibition percentages of DPPH radicals.

Table 3: The percentages of the previously identified active ingredients from *fenugreek* oil.

Compound	Compound Conc (%)	Reference
Linoleic acid	42.80%	(Kulkarni et al., 2022)
Linolenic acid	26.15%	
Oleic acid	14.40%	
Neryl acetate	17.3%	
Camphor	16.3%	
β-Pinene	15.05%	
β-caryophyllene	14.63%	
2,5-dimethyl pyrazine	6.14%	

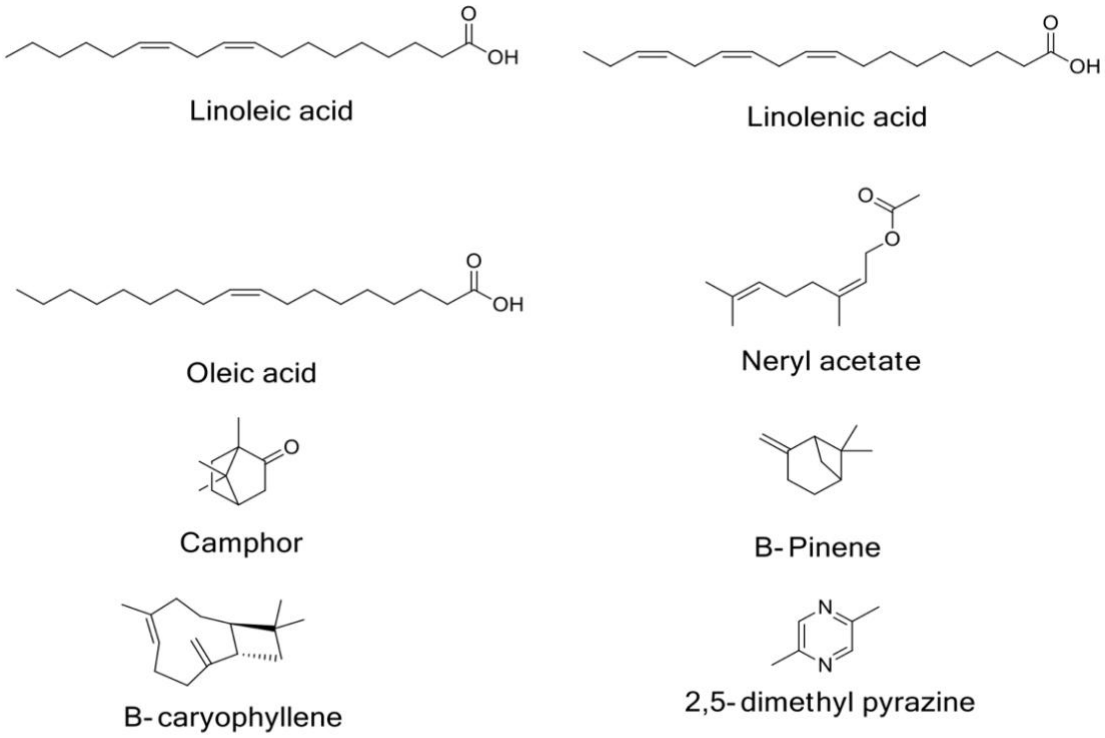

Figure 3: The chemical structures of the previously identified active ingredients from *fenugreek* oil.

Table 4: The percentages of the previously identified active ingredients from date oil.

Compound	Compound Conc (%)	Reference
Lauric acid	17.8%	
Myristic acid	ND	
Palmitic acid	ND	
Palmitoleic acid	ND	
Oleic acid	ND	(Visuvanathan et al., 2022)
Linoleic acid	15%	
Linolenic acid	8%	
Tocopherols	ND	
Phytosterols	ND	

*ND: Not determined

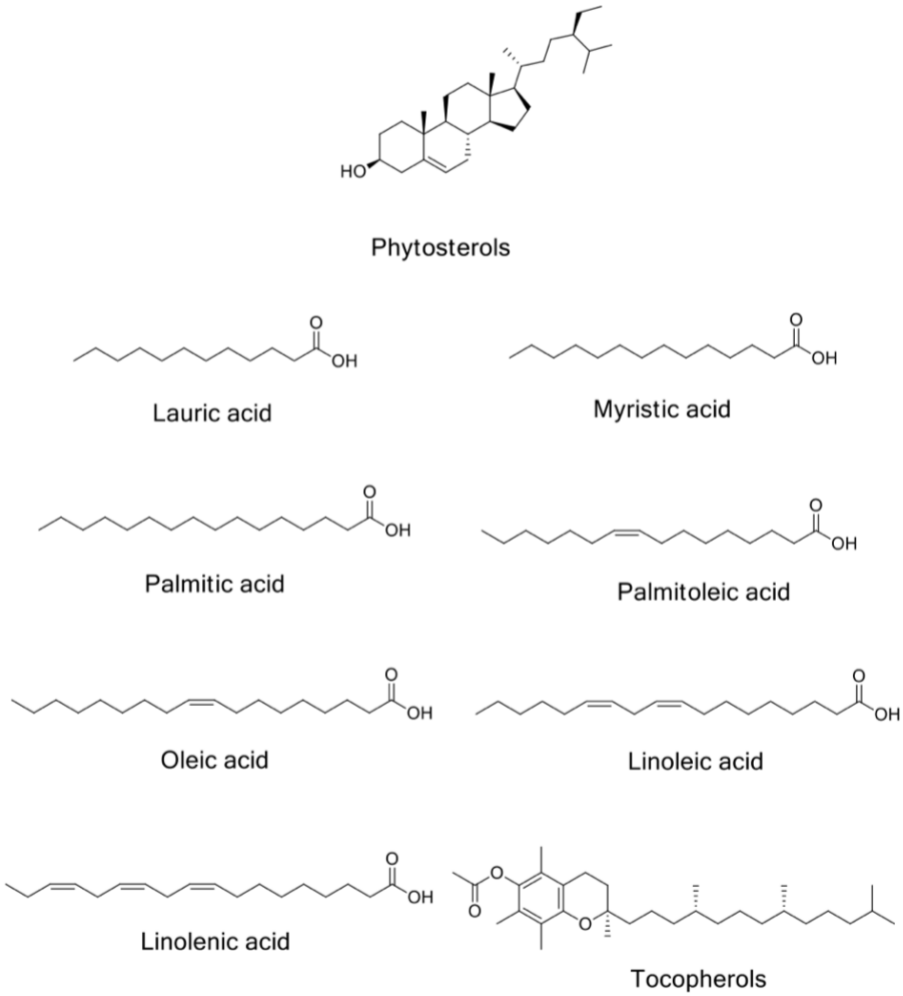

Figure 4: The chemical structures of the previously identified active ingredients from date oil.

Computational Investigations Using PASS Online:

The eight major phytoconstituents in *fenugreek* are linoleic acid, linolenic acid, oleic acid, neryl acetate, camphor, β-Pinene, β-caryophyllene, and 2,5-dimethyl pyrazine. Moreover, the nine major phytoconstituents in date are lauric acid, myristic acid, palmitic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid, tocopherols, and phytosterols, were investigated for evaluating the antioxidant activity by using PASS online program. The PASS Online web server was employed to forecast the antioxidant potential of the seven *tentatively identified fenugreek* and date oil metabolites. As indicated in **Table 5** for fenugreek oil and **Table 6** for date oil, the compounds with the highest P_a and lowest P_i values are more likely to experimentally have the highest antioxidant activity.

For *fenugreek* oil, neryl acetate possesses the highest antioxidant activity, with a P_a value of 0.865, followed by oleic acid, with a P_a value of 0.853. The remaining metabolites showed lower Pa values, which suggests lower antioxidant activity. On the other hand, β-caryophyllene possesses the lowest antioxidant activity, with a P_a value of 0.358.

Table 5: The prediction of antioxidant activity of identified metabolites from *fenugreek* oil.

Biological Activities for *Fenugreek* Metabolites	Antioxidant activity (Protein-disulfide reductase (glutathione) inhibitor)	
	P_a	P_i
Linoleic acid	0.831	0.007
Linolenic acid	0.775	0.012
Oleic acid	0.853	0.005
Neryl acetate	0.865	0.005
Camphor	0.622	0.060
β-Pinene	0.471	0.082
β-caryophyllene	0.358	0.160
2,5-dimethyl Pyrazine	0.549	0.052

P_a: Probability of being active. P_i: Probability of being inactive

As for the date oil, lauric acid, myristic acid, and palmitic acids have the highest antioxidant activity with a P_a value of 0.884, followed by palmitoleic acid and oleic acid with a P_a value of 0.853. The remaining metabolites showed lower Pa values, which suggests lower antioxidant activity. On the other hand, Tocopherols possess the lowest antioxidant activity with a P_a value of 0.294. Therefore, date oil and *fenugreek* oil have almost the same range of antioxidant activity. However, date oil may demonstrate a slightly higher range. As such, the combined oils should possess nearly a similar range of activity.

Table 6: The prediction of antioxidant activity of identified metabolites date oil.

Biological Activities for Date Metabolites	Antioxidant activity (Protein-disulfide reductase (glutathione) inhibitor)	
	P_a	P_i
Lauric acid	0.884	0.004
Myristic acid	0.884	0.004
Palmitic acids	0.884	0.004
Palmitoleic cid	0.853	0.005
Oleic acids	0.853	0.005
Linoleic acid	0.831	0.007
Linolenic acid	0.775	0.012
Tocopherols	0.294	0.235
Phytosterols	0.796	0.010

P_a: Probability of being active. P_i: Probability of being inactive

DISCUSSION

Integrating the outcomes of both the DPPH experiment and the *in silico* analysis provides crucial insights into the antioxidant properties of *fenugreek* oil and date palm oil, both individually and in combination. The DPPH analysis demonstrates the impressive antioxidant activity of *fenugreek* oil, as evidenced by its notable IC50 value of 9.95 mg/ml. This potency aligns with previous research attributing *fenugreek* oil's efficacy to its composition, notably enriched with palmitic acid and phytol, as detected through GC-MS analysis (Akbari et al., 2019). Similarly, date palm oil emerges as a potent antioxidant, boasting an IC50 value of 9.70 mg/ml. Its richness in antioxidants such as gallic, protocatechuic, p-coumaric, and ferulic acid (Mrabet et al., 2020) highlights its efficacy in neutralizing free radicals and mitigating oxidative stress.

Moreover, the *in vitro* results from the DPPH assay validate the *in silico* predictions, suggesting that date palm oil may possess a slightly higher range of antioxidant activity than *fenugreek* oil. However, both oils demonstrate comparable levels of antioxidant potential when mixed. Notably, while showing promise, the combination of *fenugreek* and date palm oils presents a surprising revelation. Despite both oils' strong individual antioxidant activities, their combined potency falls short of expectations, as indicated by an IC_{50} value of 14.2804 mg/ml. This unexpected outcome suggests a potential lack of synergistic effects in this assay, raising questions regarding the optimal mixing ratios for maximizing antioxidant effects.

When assessing the antioxidant potential of natural oils, such as those derived from *fenugreek* and dates, using computational models, notable discrepancies between predicted and experimental findings emerge. The predicted antioxidant activities of various metabolites from *fenugreek* and date oils provide intriguing insights, particularly concerning tocopherols. For instance, tocopherols in date oil are predicted to exhibit low antioxidant activity, with a P_a of 0.294 and a P_i of 0.235 (Table 6). This suggests that, when modeled computationally, tocopherols may not significantly contribute to antioxidant activity. Similarly, other metabolites, such as β-caryophyllene in fenugreek oil, are also predicted to show reduced activity, with a P_a of 0.358 (Table 5).

However, computational predictions often fail to align with the results observed in experimental settings. Despite the low predicted antioxidant activity of tocopherols, these compounds are widely recognized for their potent antioxidant properties, particularly their role as lipid-soluble antioxidants that neutralize free radicals and protect cellular membranes from oxidative damage (Traber & Stevens, 2011; DellaPenna & Mullet, 2016). The discrepancy between computational models and experimental outcomes may stem from the inherent complexity of *in vitro* conditions, which predictive algorithms cannot fully encapsulate. Tocopherols may exhibit synergistic interactions with other bioactive compounds in fenugreek and date oils, enhancing their overall antioxidant capacity (DellaPenna & Mullet, 2016; Al-Dosary & Al-Mashhadi, 2020).

This concern extends to other metabolites, such as linoleic and oleic acids, which are predicted to possess moderate to high antioxidant activity in both fenugreek and date oils (Tables 5 and 6). However, their effectiveness may vary under *in vivo* conditions. These discrepancies underscore the importance of not relying solely on computational predictions and emphasize the need for experimental validation to accurately characterize the antioxidant properties of natural plant oils (Ahmed & Farooq, 2021).

While both fenugreek and date oils have individually demonstrated considerable antioxidant activity, there remains a notable gap in the literature concerning the combined antioxidant effects of these two oils. Previous

research has predominantly concentrated on the individual properties of these oils, with limited attention given to their potential synergistic effects when used together. The present study aims to address this gap by investigating the combined antioxidant activity of fenugreek and date oils through both *in vitro* and *in silico* methods. This dual approach will provide a more nuanced understanding of how the metabolites in these oils may interact and synergistically enhance their antioxidant effects, thereby offering valuable insights into potential natural therapeutic strategies for combating oxidative stress and related diseases (Ahmed & Farooq, 2021).

Further investigation is needed into the specific mechanisms underlying these oils' antioxidant properties. For instance, a deeper analysis of the molecular interactions between the bioactive compounds in fenugreek and date palm oils could shed light on their combined antioxidant effects. Additionally, investigating the influence of factors such as extraction methods and environmental conditions on the antioxidant activity of the oils may provide valuable insights into optimizing their efficacy.

These findings emphasize the complexity of interactions among compounds within the oils and the importance of optimizing their ratios to achieve desired antioxidant effects. A significant variable in determining the antioxidant activity of oils is the ratio in which they are mixed. According to studies, mixing oils in particular ratios can have synergistic effects, meaning that the total antioxidant activity of the mixture is higher than that of the separate oils. On the other hand, some ratios can cause antagonistic interactions, which would reduce the antioxidant capability overall. For example, studies showed that altering the blending ratios can improve the antioxidant qualities of oil mixtures by increasing their stability and oxidative resistance (Mollica et al., 2020). Careful testing with various mixing ratios is necessary to find combinations that optimize antioxidant activity. By exploring the details of these interactions, researchers can unlock promising avenues for using *fenugreek* and date palm oils in various industries, including pharmaceuticals, cosmetics, and food.

CONCLUSION

Fenugreek and date palm oils individually demonstrate substantial antioxidant activity, as determined from the *in vitro* DPPH experiment and the *in silico* analysis. However, when combined, these oils exhibit a reduced antioxidant effect. The *in silico* analysis suggests that date palm oil may possess a slightly higher antioxidant activity than *fenugreek* oil. However, both oils demonstrate similar levels of antioxidant potential when mixed. These results highlight the necessity of comprehending the interplay of compounds within the oils and optimizing their mixed ratios to achieve the desired antioxidant effects. Further investigation into the specific mechanisms governing the antioxidant activities of these oils is essential, offering promising avenues for their utilization in pharmaceuticals, cosmetics, and food industries.

Conflict of interest statement

We declare that we have no conflict of interest.

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