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Beyond Total Phenolics: Antioxidant and α-Amylase Inhibitory Profiles of Foeniculum Vulgare Extracts and Solvent Fractions

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ABSTRACT: This study investigated the bioactivity profiles of *Foeniculum vulgare* extracts and fractions obtained from different geographical origins and extraction conditions. Extraction yield, total phenolic content (TPC), total flavonoid content (TFC), DPPH radical-scavenging activity, FRAP reducing capacity, and α-amylase inhibitory activity were evaluated, and the relationships among these parameters were further explored using Pearson correlation and principal component analysis (PCA). The results revealed marked variability among the investigated samples. TPC and TFC showed a relatively strong positive correlation ($r = 0.695$), whereas their associations with α-amylase inhibition were weak ($r = -0.323$ and -0.298, respectively), indicating that total phenolic and flavonoid contents alone did not adequately explain the enzyme-inhibitory response. Distinct antioxidant profiles were also observed between DPPH and FRAP assays; notably, FME exhibited high reducing capacity (FRAP = 0.900 TEAC) and strong radical-scavenging activity (DPPH-EC_{50} = 0.0495), while showing only 6% α-amylase inhibition. In contrast, DMD, SMD, and FMD displayed the highest α-amylase inhibitory activities, with values of 23.84%, 20.50%, and 18.65%, respectively. PCA provided an integrated view of these relationships, with the first two components explaining 65.442% of the total variance and revealing distinct associations among quantitative phenolic/flavonoid descriptors and biological responses. Overall, the findings demonstrate that *F. vulgare* extracts and fractions possess diverse and assay-dependent bioactivity profiles, and that their biological properties cannot be predicted solely from global phenolic or flavonoid abundance. Fractions exhibiting promising combined antioxidant and α-amylase inhibitory properties may therefore represent suitable candidates for further chemical characterization and identification of the metabolites underlying the observed activities.

1. INTRODUCTION

The increasing interest in plant-derived bioactive compounds has been stimulated by the need to identify natural sources endowed with multifunctional biological properties and potential applications in the food, pharmaceutical and nutraceutical fields. Among these compounds, phenolic constituents have received particular attention because of their broad range of biological activities, particularly antioxidant and enzyme-inhibitory effects (Albuquerque et al., 2021; Sun et al., 2020). Consequently, plant extracts rich in phenolic compounds are frequently investigated for their antioxidant and enzyme-inhibitory potential, while extraction conditions can influence the recovery and overall content of phenolic constituents (Shi et al., 2022).

However, total phenolic content (TPC) provides only a global estimate of phenolic abundance and may not fully reflect differences in biological responses. Reported relationships between TPC and antioxidant activity can vary among plant extracts and according to the assay employed, suggesting that total phenolic abundance alone may not be sufficient to explain functional activity (Skotti et al., 2014; Khiya et al., 2021).

Foeniculum vulgare Mill. (Apiaceae), commonly known as fennel, is an aromatic and widely distributed medicinal plant that has attracted considerable scientific interest because of its diverse phytochemical composition and reported biological properties. Different parts of the plant contain phenolic acids, flavonoids and other secondary metabolites that may contribute to antioxidant, antimicrobial, anti-inflammatory and enzyme-modulating effects (Rafieian et al., 2024; Khammassi et al., 2022). However, the phenolic characteristics and biological activities of *F. vulgare* may vary according to geographical origin, environmental conditions, plant organ, developmental stage and extraction method. Previous studies have reported differences in phenolic content and composition among fennel populations from different geographical origins and among different plant parts and developmental stages (Ferioli et al., 2017; Khammassi et al., 2022). Furthermore, extraction method and solvent selection can influence the recovery of phenolic constituents and the antioxidant properties of the resulting extracts (Malin et al., 2022). Against this background, antioxidant activity and α-amylase inhibition provide complementary functional perspectives for evaluating plant extracts. Antioxidant activity can be assessed through assays based on different reaction mechanisms, while α-amylase inhibition is relevant to the evaluation of the potential of plant extracts to modulate carbohydrate digestion (Sun et al., 2020). Thus, examining TPC and TFC together with complementary antioxidant assays and α-amylase inhibition may provide a broader assessment of the functional properties of *F. vulgare* extracts.

The present study investigated wild *F. vulgare* inflorescences collected from seven geographical locations in Algeria. Crude extracts were obtained using two extraction systems and subsequently fractionated by liquid–liquid partitioning. The resulting extracts and fractions were evaluated for total phenolic and flavonoid contents, antioxidant activities and α-amylase inhibition, with the aim of assessing how geographical origin, extraction system and fractionation affect phenolic contents, antioxidant activities and α-amylase inhibitory activity.

2. MATERIALS AND METHODS

2.1. Samples Collection and Preparation

The aerial parts (umbels) of *F. vulgare* were collected at the anthesis stage in September–October from seven sites in Algeria: Oued Morra, Ain Madhi, El Assafia, El Ghicha, Aflou, Frenda and Ain Maabed. The geographical characteristics of the collection sites are presented in Table 1. Botanical identification was carried out by Dr. Hadjira Benmebarek at the higher school of teachers of Laghouat.

Table 1: Samples Geographical Characterization

Locality	Longitude (°E)	Latitude (°N)	Altitude (m)
Aflou (A)	2.0973	34.1139	1400
Oued Morra (O)	2.3167	34.1667	1303
Ain Madhi (M)	2.3011	33.7939	987
El Assafia (S)	2.9833	33.8333	755
El Ghicha (G)	2.1500	33.9333	1156
Ain Maabed (D)	3.1294	34.8048	1038
Frenda (F)	1.050	35.0667	1064

2.2. Preparation of Extracts

Directly after collection, the flowers were carefully cleaned, sliced, and air-dried at room temperature (25 °C) for several days until complete dehydration. Then, grounded into fine powder.

Phenolic compounds were extracted from the dried flower powder by cold maceration using methanol/water (80:20, v/v) and acetone/water (70:30, v/v), following slight modifications to the method described in [25]. Briefly, 10 g of the powdered sample was extracted with 100 mL of each solvent mixture at room temperature in the dark for 24 h, with intermittent shaking. The extraction was repeated twice using fresh solvent each time. The resulting filtrates were combined and concentrated under reduced pressure to remove methanol or acetone. The concentrated aqueous extracts were first subjected to liquid–liquid partitioning with n-hexane to remove non-polar compounds, such as lipids and pigments. The remaining aqueous phase was subsequently partitioned successively with dichloromethane and ethyl acetate. The dichloromethane and ethyl acetate phases were collected separately, to obtain two fractions (dichloromethane and ethyl acetate fractions) for each extract.

After evaporation of the organic solvents, the dried dichloromethane (DCM) and ethyl acetate (AE) fractions were weighed, and their yields were calculated relative to the initial dry plant material according to the following equation. then, the dried residues were reconstituted in absolute methanol and stored at −4 °C until further analysis.

Yield (%) = [Mass of dried fraction (g) / Mass of dry plant material (g)] × 100

2.3. Determination of Total Phenolic Content

The total phenolic content (TPC) of *F. vulgare* extracts was determined using a modified Folin–Ciocalteu method (Singleton and Rossi, 1965). Gallic acid was used as the standard.

Different concentrations of gallic acid (0.03–0.3 mg/mL) were used to construct the calibration curve. Briefly, 100 µL of gallic acid solution was mixed with 500 µL of Folin–Ciocalteu reagent solution (10% v/v), vortexed, and incubated for 5 min. 2 mL of 5% sodium carbonate (Na_2CO_3, w/v) was added and the mixture was vortexed again. Following a 30 min incubation at room temperature (25 °C), absorption was measured at 760 nm using a spectrophotometer. The same procedure was applied to the plant extracts, replacing gallic acid with the respective extract solutions. The TPC was calculated from the gallic acid calibration equation, ($A = 3.2111C$; $R^2 = 0.9976$), and expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW. All analyses were performed in triplicate.

2.4. Determination of Total Flavonoids Content

The total flavonoid content (TFC) was determined spectrophotometrically based on the formation of a flavonoid–aluminum complex, following the method described by Djeridane et al. (2006). Rutin was used as the reference standard to construct the calibration curve, and the results were expressed as milligrams of rutin equivalents per gram of dry weight (mg RE/g DW). Briefly, 1 mL of the sample solution was mixed with 1 mL of 2% (w/v) $AlCl_3$ prepared in methanol. The mixture was vortexed and incubated at room temperature for 15 min, after which the absorbance was measured at 430 nm using a spectrophotometer. The same procedure was applied to the plant extracts, replacing Rutin with the respective extract solutions. All tests were performed in triplicate.

2.5. Antioxidant activity

2.5.1. 2,2-Diphenyl-1-picrylhydrazyl free radical scavenging activity

DPPH radical-scavenging activity was evaluated according to Ani et al. (2006), with slight modifications. Briefly, 1 mL of 250 µM DPPH solution was mixed with 1 mL of each fraction at different concentrations and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm against the corresponding blank. All measurements were performed in triplicate. The inhibition percentage was calculated as:

$$\text{Inhibition } \% = \frac{A_0 - A_s}{A_0} \times 100$$

Where A_0 is the absorbance of the control and A_s is the absorbance of the extract.

EC_{50} values were determined from the corresponding inhibition–concentration curves.

2.5.2. Ferric-Reducing Antioxidant Power (FRAP)

The FRAP assay was performed according to the method established by Benzie et al. (1996). with some modifications. The FRAP working solution was prepared by combining 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ dissolved in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a volumetric ratio of 10:1:1. To perform the assay, 100 µL of the extract or reference standard (Trolox

and ascorbic acid) was mixed with 1mL of FRAP reagent, vortexed, and incubated for 10 min in the dark at room temperature. Absorbance was measured at 593 nm against a blank.

A Trolox calibration curve was established over the concentration range of 0–0.15 g/L, showing a strong linear relationship (R^2 = 0.998). The FRAP values of the extracts were calculated from the Trolox calibration equation and expressed as micromoles of Trolox equivalents per gram of dry weight (µmol TE/g DW).

2.6. In vitro α-amylase inhibitory assay

The inhibitory effect of the samples on porcine pancreatic α-amylase (EC 3.2.1.1) was assessed according to the procedure originally described by Dygert et al. (1965), with appropriate modifications. Briefly, the reaction was initiated by preparing a mixture containing 100 µL of soluble starch, 200 µL of 50 mM phosphate buffer (pH 7.0) supplemented with 6 mM NaCl, and 100 µL of the sample at a fixed concentration. This mixture was pre-incubated at 37 °C for 10 min. Subsequently, 100 µL of the enzyme solution (porcine pancreatic α-amylase, 10 IU/mL) prepared in phosphate buffer was added, followed by incubation at 37 °C for an additional 2 min. The reaction was then developed by adding 1 mL of cupric reagent containing glycine (0.21 M), Na_2CO_3 (0.38 M), and $CuSO_4$ (1.8 mM), followed by 1 mL of neocuproine solution (4.9 mM). The reaction tubes were immediately transferred to a boiling water bath (100 °C) for 10 min. After cooling to room temperature, 3 mL of distilled water was added to each tube, and the absorbance was recorded at 452 nm.

The percentage inhibition was calculated according to the following equation:

$$\mathrm{PI} = \frac{A_0 - A_1}{A_0} \times 100$$

where (PI) represents the percentage of inhibition, (A_0) is the absorbance measured for the control without the tested fraction, and (A_1) is the absorbance recorded in the presence of the tested fraction.

2.7. Statistical Analysis

Principal component analysis (PCA) was achieved to explore the relationships between the samples and the measured variables, including extraction yield, total phenolic content (TPC), total flavonoid content (TFC), FRAP antioxidant activity, DPPH-EC_{50}, and α-amylase inhibitory activity. The analysis was based on standardized data, and the first two principal components (F1 and F2) were used to visualize the distribution of the samples and the contribution of the variables. A PCA biplot was generated to examine the relationships between the samples and variables and to identify the variables contributing most to sample discrimination.

3.RESULTS AND DISCUSSIONS

3.1. Extraction Yield and Phenolic and Flavonoid Contents

It can be seen from Table 2 that the extraction yields (0.123–3.890%), the TPC (0.091–6.308 mg GAE/g DW), and TFC (0.039–6.390 mg RE/g DW) values were highly variable among the samples analyzed. High extraction yields were found for the acetonic DCM fractions of Oued Mourra and Aflou, as well as for the methanolic DCM fraction of Ain Madhi, which indicates the impact of the solvent choice on extraction efficiency (Frosi et al., 2021; E. Gil-Martín et al., 2021). At the same time, higher amounts of both types of phytochemicals were found in EA fractions of the samples tested, with the highest TPC in the acetonic EA fraction of Oued Mourra (6.308 mg GAE/g DW) and the highest TFC in the methanolic EA fraction of Assafiya (6.390 mg RE/g DW). It corroborates the efficiency of the use of moderately polar solvents like ethyl acetate in the extraction of the relatively polar phenolics from North African plants (Boussoussa et al., 2014; El Kamari et al., 2024; Hamdi et al., 2024).

Table 2: Table X. Extraction yield, total phenolic content, and total flavonoid content of *Foeniculum vulgare* extracts and solvent fractions from different Algerian regions.

	Site	**Yield (%)**		**TPC (mgGAE/gDW)**		**TFC (mgRE/gDW)**	
		Methanolic extract	Acetonic extract	Methanolic extract	Acetonic extract	Methanolic extract	Acetonic extract
DCM	Ain Madi	2.58	2.24	1.083±0.220	1.142±0.102	0.148±0.01	0.196±0.014

	Assafiya	0.346	1.67	0.418±0.039	0.726±0.067	0.387±0.009	0.229±0.010
	Aflou	0.29	3.2	0.197±0.010	1.728±0.264	0.185±0.029	0.480±0.02
	Al-Ghishah	0.21	2.61	0.132±0.015	0.456±0.028	0.100±0.012	0.358±0.032
	Oued Mourra	0.123	3.89	0.091±0.010	1.342±0.266	0.039±0.000	0.437±0.024
	Ain Maabad	0.439	1.37	0.285±0.038	5.471±0.349	0.228±0.12	0.781±0.07
	Ferenda	2.145	1.63	1.115±0.183	1.059±0.034	0.268±0.013	0.529±0.040
Ethyl acetate fraction	Ain Madi	0.597	2.4	1.767±0.110	0.984±0.213	1.743±0.01	3.264±0.072
	Assafiya	2.159	1.82	3.648±0.403	2.269±0.353	6.390±0.086	1.306±0.015
	Aflou	1.317	1.87	2.304±0.270	1.290±0.209	2.792±0.131	0.607±0.023
	Al-Ghishah	0.591	2.55	0.738±0.083	0.855±0.138	0.212±0.035	0.109±0.009
	Oued Mourra	0.528	1.45	1.478±0.124	6.308±1.214	0.422±0.021	5.735±0.143
	Ain Maabad	1.194	1.81	2.662±0.353	0.778±0.183	2.770±0.071	0.248±0.033
	Ferenda	0.884	2.48	1.529±0.152	1.525±0.136	1.626±0.053	1.468±0.031

The presence of a positive relationship between TPC and TFC (r = 0.695) means that those fractions that were rich in total phenolics usually had high amounts of flavonoids. Nevertheless, a moderate nature of such relation suggests that flavonoids did not explain all variations in TPC, hence the different degree of contribution of flavonoid and non-flavonoid phenols. Such phenomenon might be due to the different principles of measurements using the Folin-Ciocalteu and $AlCl_3$ methods (Raposo et al., 2024; Nicolescu et al., 2025).

3.2. Antioxidant activity (DPPH–EC_{50} and FRAP–TEAC)

The antioxidant activity of analyzed fractions was very different depending on the type of fraction and the test used (Table 3). In the DPPH test, EA fractions showed stronger radical-scavenging activity, which was shown by their lower EC_{50} values.

Table 3: Table X. Antioxidant activity of *Foeniculum vulgare* samples from different Algerian regions expressed as FRAP-TEAC and DPPH-EC_{50} values.

	TEAC-FRAP				**EC_{50}-DPPH (g/L)**			
	Methanolic extract		Acetonic extract		Methanolic extract		Acetonic extract	
	DCM	EA	DCM	EA	DCM	EA	DCM	EA
Ain Madi	0.153±0.005	0.52±0.01	0.073 ±0.002	0.549 ±0.007	-	0.032±0.001	2.5±0.003	0.052±0.001
El Assafia	1.42±0.02	0.21±0.003	0.095 ±0.002	0.196 ±0.016	0.231±0.009	0.094±0.094	0.61±0.0009	0.129±0.001
Aflou	0.141±0.009	0.26±0.007	0.056 ± 0.001	0.252 ± 0.002	0.30±0.002	0.160±0.009	3.434±0.002	0.272±0.002
El Ghicha	0.128±0.013	0.90±0.0005	0.05 ± 0.001	0.0014±0.0001	0.36±0.0062	0.099±0.06	2.29±0.02	3.05±0.02

Oued Mourra	0.108±0.012	0.14±0.002	0.035 ± 0.005	0.170 ±0.008	0.246±0.01	0.049±0.05	2.773±0.01	0.114±0.0025
AinMaabad	0.118±0.003	0.14±0.005	0.149 ± 0.005	0.181 ±0.001	0.36±0.1	0.01±0.004	3.53±0.01	0.74±0.005
Ferenda	0.122±0.0007	1.8±0.003	0.082 ±0.001	0.130 ±0.012	0.30±0.004	0.099±0.5	0.67±0.0003	0.048±0.0005
Vit C	1.580 ± 0.02				0.0089			

The antioxidant activity of analyzed fractions was very different depending on the type of fraction and the test used (Table 3). In the DPPH test, EA fractions showed stronger radical-scavenging activity, which was shown by their lower EC_{50} values. The smallest EC_{50} values were found for the methanolic EA fractions from Ain Maabad (0.010 ± 0.004 g/L) and Ain Madi (0.032 ± 0.001 g/L; Tab 3). On the hand the acetonic DCM fractions usually had less activity. In contrast, the FRAP test a different activity distribution. The methanolic EA fraction from Ferenda and the DCM fraction from El Assafia displaying the highest reducing capacities. The different patterns observed in the DPPH and FRAP results can be explained by the fact that each assay works through a different chemical mechanism. (Schlesier et al. 2002; Prior et al., 2005). Therefore, the two tests may not always show the same antioxidant activity.

The correlation analysis (Table 4) showed generally that the links between phenolic content TPC and total flavonoid TFC content were not strongly related to antioxidant activity. Total phenolic content had a link with DPPH (r = 0.112) and a weak link with FRAP (r = -0.092). TPC had a negative link with DPPH (r = -0.327) and almost a negligible correlation with FRAP (r = 0.058). These results mean that the TPC and f TFC alone did not adequately explain the effects. This might be because the actual types and specific features of the components play a big role (Aouadi et al. 2025; Shan et al., 2019). Also, differences in how the antioxidant tests work could be another reason, for these links. Some phenolic parts can have abilities to stop free radicals and to transfer electrons (Chen et al., 2020).

3.3. α-amylase inhibitory activity

Inhibition of α-amylase can be taken as an important in vitro method to study the potential of plant extracts to inhibit the breakdown of starch into glucose upon consumption of carbohydrates. The breakdown of starch occurs through α-amylase into oligosaccharides and is converted to absorbable monosaccharide units through intestinal α-glucosidase enzymes. The inhibition of such enzymes has, therefore, been suggested to be a means of reducing postprandial hyperglycemia (Papoutsis et al., 2021; Kashtoh & Baek, 2023).

Table 4: α-Amylase inhibitory activity (%) of *Foeniculum vulgare* samples from different Algerian regions.

	I (%)			
	Methanolic extract		**Acetonic extract**	
	DCM	**EA**	**DCM**	**EA**
Ain Madi	4.80	0	0	0
El Assafia	20.50	0	0	0
Aflou	0	0	0.80	0
El-Ghishah	0	13.46	0	11.50
Oued Mourra	0	0	0	0
Ain Maabad	23.84	0	0	15.00
Ferenda	18.65	6	0	5.50
Acarbose	18			

The inhibitory activity of α-amylase was different for the extracts and fractions despite their identical concentrations during testing. DMD displayed the highest inhibitory activity (23.84%), followed by SMD (20.50%) and FMD (18.65%). The mentioned results were comparable to the value of acarbose (18%) or higher than it, whereas other samples displayed low or no inhibition.

The differences between fractions and sites may be related to variations in phytochemical composition and the selective recovery of bioactive compounds during extraction and fractionation. Environmental conditions can influence the production and accumulation of bioactive compounds in plants (Pant et al., 2021), while geographical differences may also affect phenolic composition and enzyme-inhibitory activity (Mezni et al., 2026). Solvent characteristics and extraction conditions can further influence the recovery of compounds with different inhibitory properties (Papoutsis et al., 2021; Chang et al., 2021; Tlili et al., 2019).

These findings show that having greater TPC and TFC values does not necessarily lead to a stronger α-amylase inhibition activity, as evidenced by the relatively low correlation observed between them, namely TPC (r= -0.323) and TFC (r= -0.298). Similarly, there is also low correlation between antioxidant activity and α-amylase activity.

The antioxidant and α-amylase inhibition activity were found not to follow similar trends, indicating that both of them are likely to involve dissimilar molecular interactions. It could mean that both the observed inhibition effects depend more on specific compounds and their structure rather than their total amount of phenolic and flavonoids (Lam et al., 2024; Kashtoh & Baek, 2023; Peng et al., 2024). This interpretation agrees with previous findings of the α-amylase inhibition activity of phenolic extracts of medicinal plants in Algeria (Djeridane et al., 2015). These results reflect activity at the tested concentration, while IC_{50} determination would be required for a more accurate comparison of inhibitory potency.

3.4. Multivariate analysis: Principal Component Analysis

Principal component analysis (PCA) was performed to merge the data obtained on the yields of extracts, TPC, TFC, DPPH, FRAP, and α-amylase inhibitory activity, with dimensionality reduction being carried out using orthogonal components (Jolliffe and Cadima, 2016). Based on eigenvalues, it is known that F1 (eigenvalue = 2.123; 35.387% variance) and F2 (eigenvalue = 1.803; 30.055% variance) accounted for 65.442% of variance, while the contribution of F3, F4, F5, and F6 was 14.402%, 9.621%, 8.644%, and 1.891%, respectively. Therefore, the factorial plane formed by F1–F2 (Figure 3) accounted for most of the multivariate information (Liu et al., 2024; Kutlu et al., 2024).

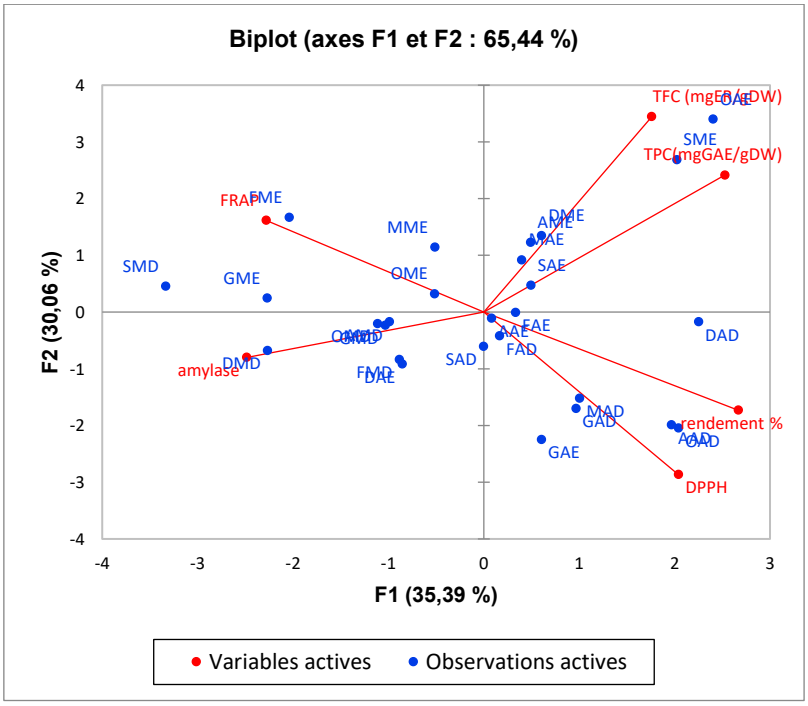

Fig3 : PCA biplot illustrating the relationships among extracts and extraction yield, TPC, TFC, DPPH-EC50, FRAP, and α-amylase inhibition on the F1–F2 factorial plane

TPC and TFC were found to have a significant relationship in the F1-F2 biplot, consistent with their high Pearson correlation coefficient (r = 0.695 ; Table 4), and showing a tendency to vary together. Their respective structural weights, however, were different: TFC dominated the structural weight of F2 (36.867%), whereas TPC had its structural weight divided into two factors, namely F1 (19.848%) and F2 (18.146%). The high cos² values of TPC (0.748) and TFC (0.870) showed that both variables were well represented on the factorial plane. In essence, even though positively correlated, these global spectrophotometric measures provided different multivariate aspects, since they do not provide information about specific phenolic compounds. (Liu et al., 2024).

There were similarities between the vectors of yield and DPPH-EC_{50} due to the positive correlation observed between the two vectors (r = 0.578). As mentioned earlier, low DPPH-EC_{50} corresponds to high antioxidant activities; therefore, such similarity in the orientation of these vectors is an indication of the numerical value of EC_{50} rather than antioxidant activities. DPPH had a high cos² value on F1–F2 (cos² = 0.275 and 0.460), while FRAP had an opposite orientation on F1 (−0.587), which is attributed to the difference in chemical concepts between these two antioxidant tests (Munteanu and Apetrei, 2021; Schaich et al., 2021). The inhibition of α-amylase had a high association with F1 (−0.639; contribution = 19.231%), but low representation on F2 (cos² = 0.036). In addition, weak correlation with TPC (r = −0.323) and TFC (r = −0.298) shows the lack of relationship between the level of inhibition and the total phenolic content.

Table 4 : Pearson correlation coefficients among extraction yield, TPC, TFC, DPPH-EC_{50}, FRAP, and α-amylase inhibition

Variables	Yield %	TPC(mgGAE/gDW)	TFC (mgER/gDW)	DPPH	FRAP	amylase
Yield %	**1**	**0,139**	**0,106**	**0,578**	**-0,361**	**-0,184**
TPC(mgGAE/gDW)	**0,139**	**1**	**0,695**	**0,112**	**-0,092**	**-0,323**
TFC (mgER/gDW)	**0,106**	**0,695**	**1**	**-0,327**	**0,058**	**-0,298**
DPPH	**0,578**	**0,112**	**-0,327**	**1**	**-0,332**	**-0,115**
FRAP	**-0,361**	**-0,092**	**0,058**	**-0,332**	**1**	**0,319**
amylase	**-0,184**	**-0,323**	**-0,298**	**-0,115**	**0,319**	**1**
Les valeurs en gras sont différentes de 0 à un niveau de signification alpha=0,95						

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None of the groups were distinguished by extraction solvent, fractionation solvent, or geographical location. This shows that the distribution was influenced by the variation in composition of the extracted compounds and not by one specific solvent or geographical influence (Kutlu et al., 2024; Uaila et al., 2024). Of all the samples studied, SMD had the greatest influence on F1 (19.42%), while OAE had the greatest influence on F2 (23.72%). The contribution here shows the influence of samples in building the factorial axis rather than the biological strength of the samples (Jolliffe and Cadima, 2016). In general, PCA added value to the Pearson correlation test by giving an overview of the relationship between all the measured variables (Liu et al., 2024; Lemos et al., 2024).

4.CONCLUSION

In conclusion, the present study has shown that *Foeniculum vulgare* can serve as a good source of extractable and fractionated products of different biological potential. The presence of great variability in the bioactivity profiles of samples is an example of the importance of the impact of extraction and fractionation conditions and geography of sample origin on bioactivity.

Notably, antioxidant and α-amylase inhibitory potentials were not correlated with the content of total phenolics or flavonoids, which means that neither antioxidant nor α-amylase activities can be estimated by total phenolic or flavonoid amounts.

The analysis of antioxidant activity, α-amylase inhibition, correlation coefficients, and PCA clearly demonstrates the multidimensional character of the bioactivity responses detected. Thus, fractions possessing interesting antioxidant or α-amylase inhibitory activity may be subjected to additional chromatographic and spectrometric studies for the identification of bioactive compounds.

REFERENCES

[1] Albuquerque, B.R., Heleno, S.A., Oliveira, M.B.P.P., Barros, L., Ferreira, I.C.F.R., 2021. Phenolic compounds: current industrial applications, limitations and future challenges. *Food & Function* 12, 14–29. https://doi.org/10.1039/D0FO02324H.

[2] Ani, V., Varadaraj, M.C., Naidu, K.A., 2006. Antioxidant and antibacterial activities of polyphenolic compounds from bitter cumin (*Cuminum nigrum* L.). *European Food Research and Technology* 224, 109–115. https://doi.org/10.1007/s00217-006-0295-z.

[3] Aouadi, A., Abd El-Mordy, F.M., Saoud, D.H., Mustafa, Y.A.A., Zidane, H., Belkhalifa, H., Berkane, O., Choula, S., Zohra, A.A.F., Hadjadj, S., Atoki, A.V., Djemoui, A., Messaoudi, M., 2025. Exploring antioxidant, antibacterial and anti-hemolytic activities of *Atriplex halimus* L.: Optimizing extraction conditions to enhance bioactive and pharmaceutical properties. *Natural Product Communications* 20(12). https://doi.org/10.1177/1934578X251410057.

[4] Apak, R., Güçlü, K., Demirata, B., Özyürek, M., Çelik, S.E., Bektaşoğlu, B., Berker, K.I., Özyurt, D., 2007. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules* 12(7), 1496–1547. https://doi.org/10.3390/12071496.

[5] Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry* 239(1), 70–76. https://doi.org/10.1006/abio.1996.0292.

[6] Boukada, F., Meddah, B., Soltani, F.Z., Sitayeb, S., 2023. Efficacy of the crude extract and solvent fractions of *Lavandula stoechas* L. for potential antibacterial and antioxidant capacity: an endemic medicinal plant from Algeria. *Agriculturae Conspectus Scientificus* 88(1), 29–36.

[7] Boussoussa, H., Hamia, C., Djeridane, A., Boudjeniba, M., Yousfi, M., 2014. Effect of different solvent polarity on extraction of phenolic compounds from Algerian *Rhanterium adpressum* flowers and their antimicrobial and antioxidant activities. *Current Chemical Biology* 8(1), 43–50. https://doi.org/10.2174/2212796808011411120959 50.

[8] Chang, K.A., Ley, S., Lee, M., Yaw, H., Lee, S., Chew, L.Y., Neo, Y.P., Kong, K.W., 2021. Determination of nutritional constituents, antioxidant properties, and α-amylase inhibitory activity of *Sechium edule* (chayote) shoot from different extraction solvents and cooking methods. *LWT* 151, 112177. https://doi.org/10.1016/j.lwt.2021.112177.

[9] Chen, J., Yang, J., Ma, L., Li, J., Shahzad, N., Kim, C.K., 2020. Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. *Scientific Reports* 10(1), 2611. https://doi.org/10.1038/s41598-020-59451-z.

[10] Djeridane, A., Hamdi, A., Bensania, W., Cheifa, K., Lakhdari, I., Yousfi, M., 2015. The in vitro evaluation of antioxidative activity, α-glucosidase and α-amylase enzyme inhibitory of natural phenolic extracts. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews* 9(4), 324–331. https://doi.org/10.1016/j.dsx.2013.10.007.

[11] Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., Vidal, N., 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry* 97(4), 654–660. https://doi.org/10.1016/j.foodchem.2005.04.028.

[12] Djeridane, A., Yousfi, M., Nadjemi, B., Maamri, S., Djireb, F., Stocker, P., 2006. Phenolic extracts from various Algerian plants as strong inhibitors of porcine liver carboxylesterase. *Journal of Enzyme Inhibition and Medicinal Chemistry* 21(6), 719–726. https://doi.org/10.1080/14756360600810399.

[13] Dygert, S., Li, L.H., Florida, D., Thoma, J.A., 1965. Determination of reducing sugar with improved precision. *Analytical Biochemistry* 13(3), 367–374. https://doi.org/10.1016/0003-2697(65)90327-1.

[14] El Kamari, F., El Omari, H., El-Mouhdi, K., Chlouchi, A., Harmouzi, A., Lhilali, I., El Amrani, J., Zahouani, C., Hajji, Z., Ousaaid, D., 2024. Effects of different solvents on the total phenol content, total flavonoid content, antioxidant, and antifungal activities of *Micromeria graeca* L. from Middle Atlas of Morocco. *Biochemistry Research International* 2024, 9027997. https://doi.org/10.1155/2024/9027997.

[15] Ferioli, F., Giambanelli, E., D'Antuono, L.F., 2017. Fennel (*Foeniculum vulgare* Mill. subsp. *piperitum*) florets, a traditional culinary spice in Italy: evaluation of phenolics and volatiles in local populations, and comparison with the composition of other plant parts. *Journal of the Science of Food and Agriculture* 97(15), 5369–5380. https://doi.org/10.1002/jsfa.8426.

[16] Frosi, I., Montagna, I., Colombo, R., Milanese, C., Papetti, A., 2021. Recovery of chlorogenic acids from agri-food wastes: updates on green extraction techniques. *Molecules* 26, 4515. https://doi.org/10.3390/molecules26154515.

[17] Guan, L., Long, H., Ren, F., Li, Y., Zhang, H., 2022. A structure-activity relationship study of the inhibition of α-amylase by benzoic acid and its derivatives. *Nutrients* 14(9), 1931. https://doi.org/10.3390/nu14091931.

[18] Hamdi, A., Djeridane, A., Hachani, S., Youssefi, Z., Yousfi, M., 2024. Seasonal and solvent extraction influence on phenolic contents, antioxidant activity and antidiabetic capacity of three selected herbs from Chenopodiaceae family. *Vegetos* 38(2), 542–551. https://doi.org/10.1007/s42535-023-00789-w.

[19] Jolliffe, I.T., Cadima, J., 2016. Principal component analysis: a review and recent developments. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 374, 20150202. https://doi.org/10.1098/rsta.2015.0202.

[20] Kashtoh, H., Baek, K.-H., 2023. New insights into the latest advancement in α-amylase inhibitors of plant origin with anti-diabetic effects. *Plants* 12(16), 2944. https://doi.org/10.3390/plants12162944.

[21] Khammassi, M., Mighri, H., Ben Mansour, M., Amri, I., Jamoussi, B., Khaldi, A., 2022. Metabolite profiling and potential antioxidant activity of sixteen fennel (*Foeniculum vulgare* Mill.) populations growing wild in Tunisia. *South African Journal of Botany* 148, 407–414. https://doi.org/10.1016/j.sajb.2022.05.021.

[22] Khiya, Z., Oualcadi, Y., Gamar, A., Berrekhis, F., Zair, T., El Hilali, F., 2021. Correlation of total polyphenolic content with antioxidant activity of hydromethanolic extract and their fractions of the *Salvia officinalis* leaves from different regions of Morocco. *Journal of Chemistry* 2021, 8585313. https://doi.org/10.1155/2021/8585313.

[23] Kutlu, N., Kamiloğlu, A., Abca, T.E., Yilmaz, Ö., 2024. Ultrasound-assisted deep eutectic solvent extraction of bioactive compounds from persimmon calyx. *Journal of Food Science* 89(1), 294–305. https://doi.org/10.1111/1750-3841.16849.

[24] Lam, T.P., Tran, N.N., Pham, L.D., Lai, N.V., Dang, B.N., Truong, N.N., Nguyen-Vo, S.K., Hoang, T.L., Mai, T.T., Tran, T.D., 2024. Flavonoids as dual-target inhibitors against α-glucosidase and α-amylase: a systematic review of in vitro studies. *Natural Products and Bioprospecting* 14(1), 4. https://doi.org/10.1007/s13659-023-00424-w.

[25] Liu, H., Agar, O.T., Imran, A., Barrow, C.J., Dunshea, F.R., Suleria, H.A.R., 2024. LC-ESI-QTOF-MS/MS characterization of phenolic compounds in Australian native passion fruits and their potential antioxidant activities. *Food Science & Nutrition* 12(4), 2455–2472. https://doi.org/10.1002/fsn3.3928.

[26] Magalhães, L.M., Segundo, M.A., Reis, S., Lima, J.L.F.C., 2008. Methodological aspects about in vitro evaluation of antioxidant properties. *Analytica Chimica Acta* 613(1), 1–19. https://doi.org/10.1016/j.aca.2008.02.047.

[27] Malin, V., Elez Garofulić, I., Repajić, M., Zorić, Z., Pedišić, S., Sterniša, M., Smole Možina, S., Dragović-Uzelac, V., 2022. Phenolic characterization and bioactivity of fennel seed (*Foeniculum vulgare* Mill.) extracts isolated by microwave-assisted and conventional extraction. *Processes* 10(3), 510. https://doi.org/10.3390/pr10030510.

[28] Mezni, F., Aouinti, H., Ben Belaid, N., Ayari, F., El Hadj, A., 2026. *Iris planifolia* (Mill.) T. Durand & Schinz: geographical variation in phenolic profile and antidiabetic activity. *Natural Product Research*, 1–7. https://doi.org/10.1080/14786419.2026.2669208.

[29] Munteanu, I.G., Apetrei, C., 2021. Analytical methods used in determining antioxidant activity: a review. *International Journal of Molecular Sciences* 22(7), 3380. https://doi.org/10.3390/ijms22073380.

[30] Nicolescu, A., Bunea, C.I., Mocan, A., 2025. Total flavonoid content revised: an overview of past, present, and future determinations in phytochemical analysis. *Analytical Biochemistry* 700, 115794. https://doi.org/10.1016/j.ab.2025.115794.

[31] Pant, P., Pandey, S., Dall'Acqua, S., 2021. The influence of environmental conditions on secondary metabolites in medicinal plants: a literature review. *Chemistry & Biodiversity* 18(11), e2100345. https://doi.org/10.1002/cbdv.202100345.

[32] Papoutsis, K., Zhang, J., Bowyer, M.C., Brunton, N., Gibney, E.R., Lyng, J., 2021. Fruit, vegetables, and mushrooms for the preparation of extracts with α-amylase and α-glucosidase inhibition properties: a review. *Food Chemistry* 338, 128119. https://doi.org/10.1016/j.foodchem.2020.128119.

[33] Peng, Q., Ma, Y., Wang, Z., Wang, J., 2024. Inhibition mechanism of different structural polyphenols against α-amylase studied by solid-state NMR and molecular docking. *International Journal of Biological Macromolecules* 275(Part 2), 133757. https://doi.org/10.1016/j.ijbiomac.2024.133757.

[34] Prior, R.L., Wu, X., Schaich, K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry* 53(10), 4290–4302. https://doi.org/10.1021/jf0502698.

[35] Rafieian, F., Amani, R., Rezaei, A., Karaça, A.C., Jafari, S.M., 2024. Exploring fennel (*Foeniculum vulgare*): composition, functional properties, potential health benefits, and safety. *Critical Reviews in Food Science and Nutrition* 64, 6924–6941. https://doi.org/10.1080/10408398.2023.2176817.

[36] Raposo, F., Borja, R., Gutiérrez-González, J.A., 2024. A comprehensive and critical review of the unstandardized Folin-Ciocalteu assay to determine the total content of polyphenols: the conundrum of the experimental factors and method validation. *Talanta* 272, 125771. https://doi.org/10.1016/j.talanta.2024.125771.

[37] Schaich, K.M., Tian, X., Xie, J., 2015. Hurdles and pitfalls in measuring antioxidant efficacy: a critical evaluation of ABTS, DPPH, and ORAC assays. *Journal of Functional Foods* 14, 111–125. https://doi.org/10.1016/j.jff.2015.01.043.

[38] Schlesier, K., Harwat, M., Böhm, V., Bitsch, R., 2002. Assessment of antioxidant activity by using different in vitro methods. *Free Radical Research* 36(2), 177–187. https://doi.org/10.1080/10715760290006411.

[39] Shan, S., Huang, X., Shah, M.H., Abbasi, A.M., 2019. Evaluation of polyphenolics content and antioxidant activity in edible wild fruits. *BioMed Research International* 2019, 1381989. https://doi.org/10.1155/2019/1381989.

[40] Shi, L., Zhao, W., Yang, Z., Subbiah, V., Suleria, H.A.R., 2022. Extraction and characterization of phenolic compounds and their potential antioxidant activities. *Environmental Science and Pollution Research* 29, 81112–81129. https://doi.org/10.1007/s11356-022-23337-6.

[41] Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16, 144–158. https://doi.org/10.5344/ajev.1965.16.3.144.

[42] Skotti, E., Anastasaki, E., Kanellou, G., Polissiou, M., Tarantilis, P.A., 2014. Total phenolic content, antioxidant activity and toxicity of aqueous extracts from selected Greek medicinal and aromatic plants. *Industrial Crops and Products* 53, 46–54. https://doi.org/10.1016/j.indcrop.2013.12.013.

[43] Sun, L., Wang, Y., Miao, M., 2020. Inhibition of α-amylase by polyphenolic compounds: substrate digestion, binding interactions and nutritional intervention. *Trends in Food Science & Technology* 104, 190–207. https://doi.org/10.1016/j.tifs.2020.08.003.

[44] Tlili, N., Kirkan, B., 2019. LC-ESI-MS/MS characterization, antioxidant power and inhibitory effects on α-amylase and tyrosinase of bioactive compounds from hulls of *Amygdalus communis*: the influence of the extracting solvents. *Industrial Crops and Products* 128, 147–152. https://doi.org/10.1016/j.indcrop.2018.11.014.

[45] Uaila, E., Granfeldt, Y., Khan, M., Guiamba, I., Burleigh, S., Lazarte, C.E., 2024. Variability of antioxidant compounds and capacities in four wild fruits collected in different years and locations in Mozambique. *Journal of Agriculture and Food Research* 18, 101315. https://doi.org/10.1016/j.jafr.2024.101315.

[46] Zheng, C.-D., Li, G., Li, H.-Q., Xu, X.-J., Gao, J.-M., Zhang, A.-L., 2010. DPPH-scavenging activities and structure-activity relationships of phenolic compounds. *Natural Product Communications* 5(11), 1759–1765. https://doi.org/10.1177/1934578X1000501112.