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Antioxidant potential and quality benchmarking of commercial fennel seeds

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Abstract
Foeniculum vulgare *Mill.* seeds are known for their dual use as spices and traditional medicines. Surprisingly, there is a lack of research on the quality benchmarking of the commercially available fennel seed powder that integrates antioxidant capacity and physicochemical attributes, especially in the Indian scenario. To this end, the present investigation was performed to authenticate a wide range of antioxidant and quality attributes of the fennel seed powder obtained from the Indian market to provide a rationale for its use as a functional food. Methanolic extracts of fennel seed owder were analyzed for TPC, TFC, DPPH radical scavenging capacity, and FRAP through the utilization of assay kits validated by spectrophotometry. Physicochemical properties including moisture, ash, acid-insoluble ash, volatile oil content as well as instrumental colour parameters (L*, a*, b*) were assessed by the standardized methods. The considerable antioxidant capacity of the fennel seed powder was revealed by the TPC and TFC of 9.55 ± 0.23 mg GAE g^{-1} and 7.76 ± 0.28 mg QE g^{-1}, respectively, alongside DPPH and FRAP values reaching 6.79 ± 0.24 mM Trolox equivalents g^{-1} and 16.88 ± 0.72 mM Fe^{2+} g^{-1}. The low level of moisture (7.61%) and negligible acid-insoluble ash reflected good storage stability and purity of the sample. This research has set a standard antioxidant and quality marker for commercial fennel seed powder, thus it can be considered as a viable natural, clean-label functional component, for food formulations.

Keywords: Fennel seed powder, Foeniculum vulgare, Antioxidant activity, Total phenolic content, Total flavonoid content, DPPH radical scavenging assay, Ferric reducing antioxidant power (FRAP)

Introduction
Fennel (Foeniculum vulgare *Mill.*) is a species belonging to the Apiaceae family, which is the largest and widely distributed plant family mainly in Asia, the Mediterranean region, and parts of Europe (Brahmi *et al.*, 2013; Badgujar *et al.*, 2014) [2, 1]. Historically, fennel seeds have been used not only as spices but also as digestive stimulants and folk medicine (Noreen *et al.*, 2023) [6]. Their efficacy in the treatment of diseases has been mainly attributed to the presence of volatile oils, phenolic acids, flavonoids, and other secondary metabolites which have been chemically and biologically characterized, therefore, demonstrate antioxidant, antimicrobial, and anti-inflammatory activities (Fadavi *et al.*, 2022; Noreen *et al.*, 2023) [3, 6]. In fact, oxidative deterioration caused due to food exposure to the environment is a major toil to food systems that results in loss of food quality and safety in terms of sensory, nutritional, and health properties. Phenolics and flavonoids are the most important components of the plant defense mechanism involved in the neutralization of free radicals, chelation of metal ions, and electron donation (Noreen *et al.*, 2023) [6]. Various authors have studied the antioxidant effects of fennel seed extracts by means of through *in vitro* model systems like DPPH and FRAP (Goswami & Chatterjee, 2014; Noreen *et al.*, 2023) [5, 6]. The different varieties and characteristics of the plant, its geographical origin, the time of harvest, the solvents used for extraction, and the analytical techniques employed, among other factors, contribute to the wide range of antioxidant values reported in the literature (Noreen *et al.*, 2023) [6]. Hence the lack of consistency between research findings makes it difficult to compare one another and at the same time, it indicates the necessity of a standardized method for the evaluation of fennel seed quality (Brahmi *et al.*, 2013) [2].

Besides the antioxidant capacity, the physicochemical properties such as the moisture content, ash value, volatile oil yield, mineral impurities, and color features are very important for the quality, shelf life, and powder product.ion of spices (FSSAI, 2011; Noreen *et al*., 2023) [6, 21]. Instrumental colour evaluation of commercially available fennel seed powder is scarcely reported in the literature. The present study uniquely documents quantitative L*, a*, and b* colour parameters, thereby establishing objective visual quality benchmarks for fennel seed powder. These colour attributes provide valuable indicators of raw material freshness, degree of processing, and market quality, complementing chemical and antioxidant assessments. To date, only a handful of works have investigated the association between the antioxidant potential and quality characteristics of fennel seeds in the Indian market even though India is the largest producer and consumer of spices. Fulfillment of this research gap would be of benefit to both academic and industrial sectors in the efficient utilization of fennel as a natural functional ingredient.

Objectives of the Study

- To quantify the total phenolic and flavonoid content of commercially available fennel seed powder from Indian markets;
- To evaluate its antioxidant capacity using DPPH radical scavenging and ferric reducing antioxidant power assays;
- iii) To assess key physicochemical and colour attributes relevant to quality, purity, and storage stability; and

Materials and Methods

Materials

Fennel (Foeniculum vulgare *Mill.*) seed of a commercial brand under the provisions of FSSAI was bought off the local market of Anand (Gujarat, India). Seeds were decontaminated and then ground to powder by electric grinder. To get a uniform particle size, the powdered material was passed through a standard 35-mesh sieve. The sieved powder was stored in airtight containers at refrigeration temperature until further analysis.

Preparation of Fennel Seed Extract

For extract preparation, 2.5 g fennel seed powder was added in 50 ml of methanol-water (4:1, v/v) solvent and kept in a water bath at 37°C for 24 h as described by Murtaza *et al*. (2021) and Anwar *et al*. (2009) [9,. 10]. After 24 h, the content was filtered using Whatman No. 1 filter paper to separate the solid particles. The filtrate (methanolic extract) obtained was ten times diluted and used for the determination of the total phenolic and total flavonoids content as well as antioxidant activity by DPPH assay and FRAP assay.

Determination of Total Phenolic Content

The analysis of the total phenolic content in fennel seed extract was conducted using the Folin-Ciocalteu reagent, as described by Singleton and Rossi (1965) [11]. In a test tube, 0.1 ml of fennel seed extract was taken out, and the volume was then made to 1 ml with distilled water. Subsequently, the addition of 0.5 ml of each of the diluted Folin Ciocalteau reagent (1:1) and 10 ml of 7.5 per cent sodium carbonate was carried out. The content was mixed using a vortex mixer and incubated in the dark at room temperature for 30 min. For blank preparation, distilled water was used instead of the extract. The absorbance of the samples was measured against a blank at 750 nm using a spectrophotometer. The gallic acid standard curve was prepared using a known concentration of gallic acid (10-120 µg/ml) in a test tube and volume was made up to 1 ml with distilled water.

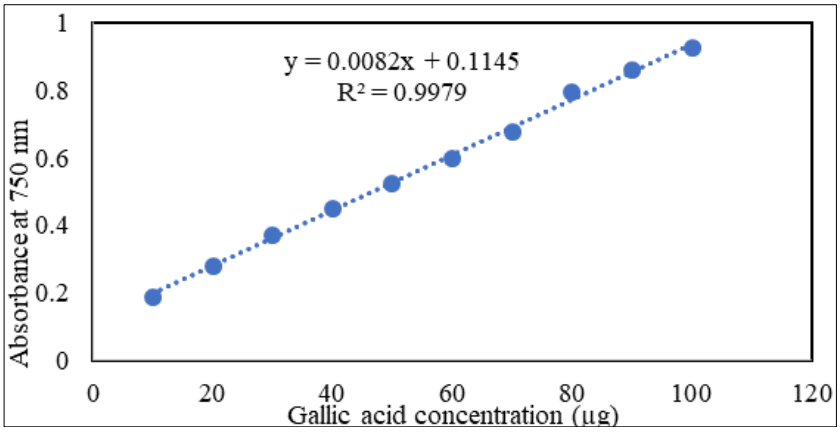

Fig 1: Gallic acid standard curve

Total phenolic content of sample was calculated based on the absorbance using equation of standard curve (Figure 1):
y = 0.0082x + 0.1145
Where, y is absorbance at 750 nm, x is concentration in µg of gallic acid
The result was expressed in terms of mg of gallic acid equivalent (GAE)/g of fennel seed powder.

Determination of Total Flavonoid Content

For the determination of total flavonoids content aluminum chloride method was used as described by Hayat *et al*., (2019) [13] with some modifications. To prepare the sample, 1 ml of the diluted fennel seed extract was measured and transferred into a 10 ml volumetric flask. Subsequently, 4 ml of distilled water was added to the flask. After that, 0.3 ml of a 5 per cent sodium nitrite solution was added to the flask. After allowing the mixture to react for 5 minutes, 0.3 ml of a 10 per cent aluminum trichloride solution was added to the flask. After that, 2 ml of a 1 M sodium hydroxide solution was added to the mixture. The volume of the flask was then made up to 10 ml using distilled water. The absorbance of the resulting solution was measured at a wavelength of 510 nm using a spectrophotometer.

In this method, a standard curve (Figure: 2) was prepared using quercetin as the standard. The flavonoid contents were then expressed as quercetin equivalents. The standard curve was prepared by using various concentrations of standard quercetin ranging from 0 to 500 µg using the same method instead of fennel seed extract in determining the total flavonoids content. Based on absorbance, the total flavonoids content of samples was calculated using equation of standard curve: y = 0.0005x + 0.0403

Where, y is absorbance at 510 nm, x is concentration in µg of quercetin. The results were expressed in terms of mg of Quercetin equivalent/g of fennel seed powder.

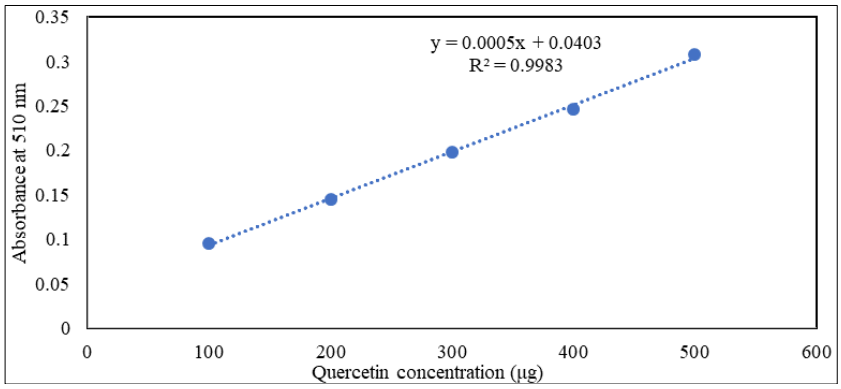

Fig 2: Quercetin standard curve

DPPH Radical Scavenging Activity

The radical-scavenging activity of spice extracts was evaluated by assessing their capacity to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, according to the procedure of Brand Williams *et al*. (1995) [12] with some modification as given by Saeed *et al*. (2009) [14]. 1 ml of a diluted fennel seed extract was transferred to a test tube. Then, 3 ml of a 0.15 mM methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was added to the test tube, and the mixture was shaken using a vortex mixer. Subsequently, the test tubes were placed in a water bath at 37°C for 30 minutes. For the control, 1 ml of methanol was used instead of the fennel seed extract. The absorbance of the reaction mixture was then measured at a wavelength of 517 nm using a spectrophotometer, with the blank serving as the reference. Radical-scavenging activity was calculated as the percentage inhibition using the following formula:

Radical-scavenging activity

(% inhibition) = [(Ac - A)/Ac] x 100

Where, Ac = absorbance of control,

A = absorbance of sample

A standard Trolox curve was prepared using various concentrations of standard antioxidant (Trolox) in the range of 0 to 40 µg and using the same instead of fennel seed extract in the method for determining the radical scavenging activity by DPPH assay. Antioxidant activity of samples was calculated based on absorbance using the equation of standard curve: y = 2.5882x - 2.9861

Where, y is per cent inhibition,

x is concentration in µg of Trolox

The results were expressed in terms of mM Trolox equivalent/g of fennel seed powder.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was measured as per the method given by Benzie and Strain (1999) [15]. Ninety microliters of diluted methanolic extract were mixed with 2.7 ml of FRAP reagent solution in 10 ml test tubes. The absorbance of the solution was measured at 595 nm using a spectrophotometer. Based on the equation determined from the standard curve, the results were expressed as mM Fe2+ per g of fennel seed powder. The standard curve was plotted by taking absorbance on Y-axis and varying from 200 to 1000 µM concentration of Fe2+ on X-axis. The reduction of absorbance was measured at 595 nm against a blank (methanol instead of sample) using a spectrophotometer.

Physicochemical Analysis

The gravimetric technique was used to determine the moisture content of fennel seed powder as described by Bukhari *et al*., 2014. Moisture content of fennel seed powder was determined by the gravimetric method using a hot air oven maintained at 102 ± 2 °C. Drying was continued until a constant weight was obtained, and moisture content was expressed on a percentage basis.

Ash and Ash Insoluble in Dilute HCl: The ash and Ash Insoluble in Dilute HCl content of fennel seed powder was measured using the method prescribed in FSSAI (2021) [17].

Volatile Oil Content

The volatile oil from fennel seed powder was extracted using the method described in FSSAI (2021) [17]. To obtain the total volatile oil insoluble in water, steam distillation was performed using Clevenger's apparatus. The apparatus consists of a round bottom flask, a separation tube, a wet condenser, and a heating mantle. First, 50 g of fennel seed powder was accurately weighed and transferred to a flask. Approximately 500 ml of distilled water was then added to the flask. For the extraction of fennel seed, the Clevenger assembly was employed, which separates the volatile oil (which is lighter than water). The apparatus was cooled to room temperature by immersing it in a water bath. The content of volatile oil was calculated using equation as follows:

$$\text{Volatile oil (\% v/w)} = \frac{\text{Volume of volatile oil (ml)}}{\text{Weight of sample (g)}}$$

The volatile oil content is expressed as aper centage (v/w).

Colour Measurement

The colour value of fennel seed powder was measured by chroma meter. First calibration of chroma meter was done on white calibration plate provided by the manufacturer along with the instrument. For the analysis of sample, the appropriate amount of powder was taken in glass petri dish and colour measurement was performed according to the three-colour coordinates (L*, a*, b*) with chromameter with in the place with minimum interference of other light sources. The a* value indicates colour ranging from red (+) to green (-), the b* value represents colour ranging from yellow (+) to blue (-), and the L* value denotes sample luminance, which ranges from black (0) to white (100).

Statistical Analysis: The obtained results were subjected to statistical analysis using Analysis of Variance (ANOVA) at a significance level of 5 per cent. For analyzing the data of each attribute under study, a statistical analysis was performed using the completely randomized design.

Results and Discussion: In the present study the authors have worked out a detailed investigation of antioxidant potential, physicochemical quality and colour attributes of fennel seed powder, which may be very useful for the development of functional foods and natural antioxidant sources. The present work is a great demonstration of how different functional; compositional and technological parameters of the material can be correlated and evaluated simultaneously.

The Total Phenolic Content and Total Flavonoid Content

The total phenolic content (TPC) and total flavonoid content (TFC) of fennel seed powder were determined as 9.55 ± 0.23 mg GAE g^{-1} and 7.76 ± 0.28 mg QE g^{-1}, respectively (Table 1). These data attest that fennel seeds are a potentially good source of phenolic antioxidants which are known for their activity in radical scavenging and inhibition of oxidative stress. The TPC value obtained has well matched with the phenolic content of fennel extracts previously reported. Liu *et al*., (2008) [18] stated a content of 7.54 mg g^{-1} phenolics in ethanolic fennel extract and Murtaza *et al*., (2021) [9] registered TPC being in the range of 1.02 - 1.26 mg GAE g^{-1} depending on the extraction solvent used. On the other hand, the TFC figures determined here are within the wide range reported by different authors, although direct comparisons at the absolute level are impeded by the fact that different factors such as cultivar, agroclimatic condition, extract solvent polarity and analytical procedures used might have been variable. With the phenolic and flavonoid contents quantified, it can be inferred that the sample of fennel seed powder analyzed in this study had a phytochemical composition matching those of reference high-grade quality fennel materials thus confirming its nutraceutical potential.

Table 1: Total phenolic and total flavonoids content of fennel seed powder

Parameter	Mean* ± SD	SEm±	CV %
Total phenolic content (mg GAE/g)	9.55± 0.23	0.071	2.46
Total flavonoids content (mg QE/g)	7.76± 0.28	0.085	3.62
* Mean± SD; n=11			

The Antioxidant Activity

The antioxidant capacity of fennel seed powder was determined by DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays with the results summarized in Table 2. The DPPH radical scavenging activity was found to be 6.79 ± 0.24 mM Trolox equivalents g^{-1}, whereas the FRAP value was 16.88 ± 0.72 mM Fe^{2+} g^{-1}. These findings highlight the potent antioxidant nature of fennel seed powder and align with previous studies that have shown a high level of radical scavenging and reducing abilities in fennel extracts and essential oils. Bilawar *et al*., (2022) [19] observed a DPPH inhibition of about 56.9% and high FRAP values for fennel oil, attributing the antioxidant properties mainly to trans-anethole, fenchone, and estragole. In the same vein, Noreen *et al*., (2023) [6] illustrated strong antioxidant activity in fennel extracts from both aqueous and organic solvents, with considerable changes depending on the polarity of the solvent used. The simultaneous use of DPPH and FRAP tests gives a comprehensive view of how antioxidants work. Saxena *et al*., (2016) [20] investigated the essential oil of ninety-one fennel samples gathered from different regions in India. They found that the essential oil content ranged between 1.0 and 3.3 per cent. The main compounds of the essential oil were anethole, α-pinene, β-pinene, myrcene, camphene, cymene, 4-allyl anisole, estragol, geranyl acetate, γ-terpinene, and p-anisaldehyde. The corroboration of the assay results in the present work indicates that both hydrogen atom transfer and single electron transfer pathways, most likely phenolic acids and flavonoids, contribute to the antioxidant activity in fennel seeds.

Table 2: Antioxidant activity of fennel seed powder

Parameter	Mean*± SD	SEm±	CV %
DPPH radical scavenging activity (mM Trolox equivalent/g)	6.79± 0.24	0.073	3.57
Ferric reducing antioxidant potential (mM Fe2+/g)	16.88 ± 0.72	0.219	4.31
* Mean± SD; n=11			

Moisture, Volatile Oil, Ash, and Ash Insoluble in Dilute HCl

The physicochemical analysis of the fennel seed powder is presented in Table 3. The moisture level was found to be 7.61 ± 0.23% (w/w), which is within the range acceptable for dried spices, thus the product is less prone to microbial attack and oxidative rancidity during storage. The values of moisture content obtained by Noreen *et al*. (2023) and Faten *et al*. (2011) [6, 21] were close to ours, thus indicating the present sample's stability. The total ash content determined on dry matter basis was 8.74 ± 0.24% and that talks about the mineral content of the fennel seeds and is also consistent with the literature data. The ash insoluble in dilute HCl was very low (0.074 ± 0.005%), which means that the presence of extraneous inorganic matter such as sand, soil, and that the whole process from harvest to packaging was well done and of good quality. The volatile oil, which is a main quality factor for fennel since it determines the aroma and also has a bioactive property, concentration was 1.56 ± 0.05% (v/w) and is within the range of values reported in the literature for the fennel seeds of the right maturity stage. According to the Food Safety and Standards Authority of India (FSSAI), 2023 specifications for fennel (Saunf) powder, the permissible limits include a moisture content not exceeding

12.0% (w/w), total ash (on a dry basis) not more than 9.0% (w/w), acid-insoluble ash (on a dry basis) not exceeding 2.0% (w/w), and a volatile oil content (on a dry basis) of not less than 1.0% (v/w). The results obtained in the present study were within the prescribed FSSAI limits, indicating good physicochemical quality and compliance with food safety standards. El-Gamal and Ahmed (2017) [22] studied the effect of different maturity stages on the essential oil content of fennel. They found that depending on the maturity level the essential oil content of the fennel varied between a low of 1.21 per cent and a high of 2.85 per cent. The essential oil content differences in the past research were explained by the variation in genotype, environmental factors, and extraction methods while the oil content we determined depicts the good quality raw material and favorable processing conditions.

Table 3: Moisture, volatile oil, ash and ash insoluble in dilute HCl content of fennel seed powder

Parameters	Mean* ±SD	SEm±	CV (%)
Moisture (per cent w/w)	7.61±0.23	0.071	3.10
Volatile oil (per cent v/w)	1.56±0.052	0.016	3.36
Ash (per cent on dry matter basis)	8.74±0.24	0.075	2.83
Ash insoluble in dilute HCl (per cent)	0.074±0.005	0.002	7.34
* Mean± SD; n=11			

Colour Characteristics

The instrumental colour analysis of the fennel seed powder (Table 4) revealed an L* value of 62.23 ± 0.62, indicating comparatively high lightness, while the negative a* value (-5.91 ± 0.16) reflects a greenish hue and the elevated b* value (33.10 ± 0.42) denotes a strong yellow component. Collectively, these values define the characteristic greenish-yellow colour associated with high-quality fennel seed powder. Fennel seed powder typically exhibits a greenish-yellow to light brown color. This hue arises primarily from natural pigments and compounds inherent in fennel seeds (Foeniculum vulgare), influenced by factors like ripeness and processing (Badgujar *et al*., 2014) [1]. Fresh fennel seeds are greenish-yellow due to residual chlorophyll, transitioning to yellowish-brown tones from carotenoids and other phytochemicals as they mature and dry into powder. Powdered form retains these traits, appearing greyish-brown to grayish-yellow, as confirmed in phytochemical screenings of seed powder (Alam *et al.*, 2019) [24]. Notably, quantitative instrumental colour data for commercially available fennel seed powder remain scarcely reported in the literature, particularly in the Indian context. In this regard, the present findings provide one of the few objective colour benchmarks for commercial fennel seed powder, offering reproducible indicators of raw material freshness, processing quality, and market acceptability. The inclusion of instrumental colour evaluation alongside antioxidant and physicochemical parameters strengthens the comprehensive quality assessment and enhances the practical relevance of the study for functional food formulation and spice standardization.

Table 4: Colour value of fennel seed powder

Colour value	L*	a*	b*
Fennel seed powder (Mean± SD)	62.23±0.62	-5.91±0.16	33.10±0.42
SEm±	0.19	0.05	0.13
CV (%)	1.00	2.78	1.27
n=11			

Conclusions

This research demonstrates that fennel (Foeniculum vulgare *Mill.*) seed powder has a good mix of antioxidant potency and other physicochemical quality properties. High contents of phenolics and flavonoids, combined with stable DPPH radical scavenging and ferric reducing antioxidant power, validate the seed powder's capability as an effective natural antioxidant. Low moisture content, minimal inorganic contamination, and desirable volatile oil levels further indicate good quality, purity, and storage stability. Collectively, these findings provide a baseline antioxidant-quality benchmark for commercially available fennel seed powder, supporting its utilization as a clean-label functional ingredient in food formulations. Future studies should focus on evaluating its performance in real food systems, processing stability, and bioavailability to further substantiate its industrial and nutritional relevance.

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