Morphological and Biochemical Characterization of *Foeniculum vulgare* Mill. (Fennel)

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Abstract

Foeniculumvulgure Mill. commonly known as Fennel (Soonf), belongs to the family Apiaceae (Umbelliferae). It is an upright, perennial herb with vast medicinal and culinary uses. It is frequently used to treat various ailments related to the digestive, endocrine, respiratory systems and reproductive systems. This study elucidates the Morphological and Biochemical variability of Fennel. The main aim was to determine the diversity of *Foeniculum vulgare*in12different varieties (accessions) based on Morphological and Biochemical characteristics. Experimental work was conducted at a Plant Genetic Resource Institute (PGRI) lab, NARC Islamabad. Firstly, 12 accessions were selected for research work. Their seeds were then sown on 15th December 2022 and grown, and the number of branches was measured between 9th March 2023 to 15th March 2023. Flowering time was also noted from initial flowering of 50% plants. After morphological characterization, fresh leaves from 12 different accessions of fennel were collected and methanol extract was prepared for evaluating the antioxidant activity of all 12 accessions, through a DPPH scavenging assay. Folin-Ciocalteu reagent was used to measure the phenolic content of 12 accessions and determination of flavonoids was determined through spectro- photometric methods. The result showed enough variation among different accesions of fennel for several yield attributing traits. It has been verified that the morphological and biochemical traits were extremely variable. The study help in effective management of crop germplasm and in improving the quality, quantity and breeding of fennel and requires advance research to reduce the effects of genetic erosion.

Keywords

Fennel (*Foeniculum vulgare*), Morphological, Total Polyphenols determinations, flavonoids, antioxidant activity.

1. INTRODUCTION

The Apiacea or Umbelliferea, also known

as a carrot family, includes the flowering plant species fennel (*Foeniculum vulgare* Mill.). It is cultivated in almost every country, especially on river, bank, and sand arid soils near the sea (Muckenstrum*et al.,* 1997). It is a perennial herb. Fennel is an upright, branching perennial herb, a glaucous green plant. It can reach a height of up to 2.5 meters with soft, feathery and hair like foliage and has hollow stems. The blooms are produces in terminal compounds umbels between 2 to 7 inches with 20 to 0 small yellow flowers on short pedicles in each umbel segment (Spellenberg, 2001). Fennel essential oils contain some important volatile compounds which enhance its campho-raceus odour. The relative concentration of fennel oil depends upon the geographical origins (Diaz Maroto*et al.,* 2006). There have been a lot of reports on oil and other activities like biological activities of oil (Uzbek *et al*., 2003); antioxidant activity (Ruberto, Baratta, Deans and Dorman, 2000); antithrombotic activity (Tongnolini*et al*., 2007);anti-inflammatory activity (Roby, 2013); antidiabetic activity (Pradhan *et al* ., 2008); antitumor activity (Lee,2004); acaricidal activity. Total 37 volatile compounds have been identified by using techniques Gas Chromatography and Gas chromatography-mass spectrometry by Guillen and Manzanos.Genetic variance in plants can be estimated by such variable tools as morphological, chemical and molecular markers. Chemical variation is a robust tool for medicinal plants characterization in relation to their morphological traits (Nsuala *et al.,* 2017).

Furthermore, the importance of fennel as a medicinal and spice plant involved the need to improve some of its agronomic characteristics, such as early ripening, larger or smaller fruits, limited growth height, higher essential oil yield and resistance to fungal diseases (Pank, 2003). Subsequently the importance of the genetic wealth for the development of improved fennel varieties is undeniable and requires further research to reduce the effects of genetic erosion. Knowledge of the morphological and chemical variable populations is important for the efficient germplasm management and long term breeding programs (Roughani *et al.,* 2018). The main aim of this study was to determine the diversity of *Foeniculumvulgare*in 12 accessions based on morphological and biochemical characteristics.

2. MATERIAL AND METHODS

The National Agriculture Research Centre (NARC) was the site of experiment. The seeds of 2 different accessions were taken, sown and grown in the field. Plants were sown in 15th December 2022. Morphological Characterization includes measurement of height of plant, and no of branches per plant among 12 accessions. For characterization of fennel, Total polyphenols, total flavonoids and antioxidant activity was determined. Fresh leaves of Fennel (*Foeniculum vulgare)* were collected from NARC (National Agriculture Research Centre) which were exported from different localities of 12 different regions grown in 12 accessions. Extract was prepared through methanol (70%) by first ground them in a pestle and mortar separately. Shaking was followed by adding methanol and grounded sample in a falcon tubes. Then the mixture was filtered through a Whatmann no .1 filter paper and placed in petri dishes for evaporation.

2.1. Determination of Total polyphenols

Folin-Ciocalteu (Fc) reagent was used to measure total phenolic content of plant extract. Gallic acid was used as a standard. Plant extract

(0.05g) was mixed with 5 ml methanol (5ml) .FC reagent (2.5ml) was taken in test tubes and plant sample (500ml) was added by micropipette. Sodium bicarbonate (2.5ml) was added and tubes were covered with aluminum foil. Test tubes was incubated in water bath at 25°c for 30 minutes. Finally the absorbance was measured at 765nm by using UV visible spectrophotometer. The same procedure was repeated for all samples and standard curve was plotted (Falleh *et al.,* 2008).

2.2. Determination of total flavonoid content

Methanol (5ml) was added in plant sample (0.001g). Vortexed them and extract (1ml) was taken by micropipette in volumetric flask (10ml). Distilled water (4ml) was added and soon after, 5% sodium nitrate solution (0.3ml) was added. Left for 5min, then 10% aluminium chloride (3ml) was added. Placed them for 6 minutes and then 1M NAOH (2ml) solution was added. Volume was made up to 10ml .Finally absorbance was checked at 510 nm by using UV Visible spectrophotometer (Faudale, Viladomat, Bastida, Poli, and Codina, 2008).

2.3. Determination of Antioxidant Activity

Plant extract (1g) was mixed with methanol (25ml). Set absorption of DPPH at 0.99nm. Firstly used methanol (70%) as a blank on UV spectrophotometer and fill second cuvette with DPPH for setting at 0.99nm. Set DPPH (3ml) was added in test tubes, Plant sample (2005Øßl) was added in test tubes by using micropipette. A test tube was placed in darkness for 20 minutes and observes color change. Absorbance was checked at 517nm UV Visible spectrophotometer (Ksouri *et al*., 2009).

3. RESULTS AND DISCUSSION

Plants were sown at 15th December, 2022 and initial flowering was observed nearly 28th February, 2023 in most of the accessions. Then,50% flowering was noticed between 9th March 2023 to 15th March, 2023.

The height and number of branches were measured. The result showed that the maximum average height gained by accession 20576 i.e., 36 while number of branches per plant accession 20810 i.e., 44.

The Fig-1 shows the graphical representation of variation between morpholo- gical characters i.e., average height of plant accessions and no of branches per plant.

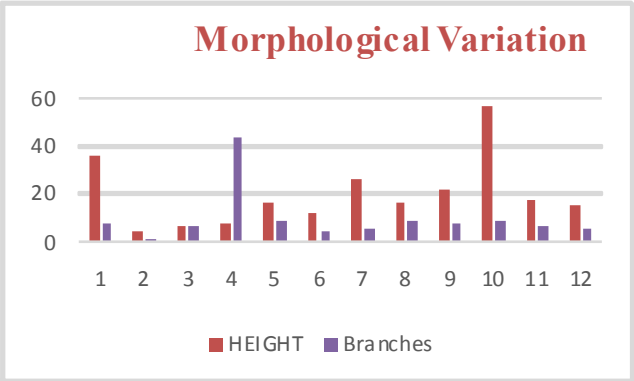

Fig-1: Graphical representation of Morphological variation between accessions

Table 1. Morphological Variation between different characters of Fennel

Accessions	Height (cm) Average	No of Branches Average	Days in Flower Initiation(15 /12/2022-28/02/2023)	50% Flowering(15/12/2022 -9/03/2023)
20575	36	8	76 days	85 days
20711	4	1	76 days	85 days
20786	6	6	76 days	85 days
20810	8	44	77 days	85 days
21396	16	9	76 days	85 days
21515	12	4	76 days	85 days
21539	26	5	76 days	85 days
21562	16	9	76 days	85 days
21757	21.7	8	76 days	85 days
21759	57	9	76 days	85 days
35858	17	6	76 days	91 days
38690	15	5	76 days	91 days

Highest amount of phenols were detected in methanolic extract of Fennel accession number **20575** i.e., 29.585. Fig-2 shows the graphical representation of total polyphenols in different accession of fennel. It has been showed that, by increasing concentration of sample, the scavenging activity of DPPH increases and increases the standard to a certain extent and had been said that scavenging activity of radical strongly depends on the extract concentration (Motalleb *et al*., 2005). It has also been observed that there is a strong relation exists between antioxidant activity and phenols. It has been reported by the (Velioglu *et al*., 1998) that there is no correlation between phenolics and antioxidant activity in plant extract (Kahkonen *et al*., 1999).

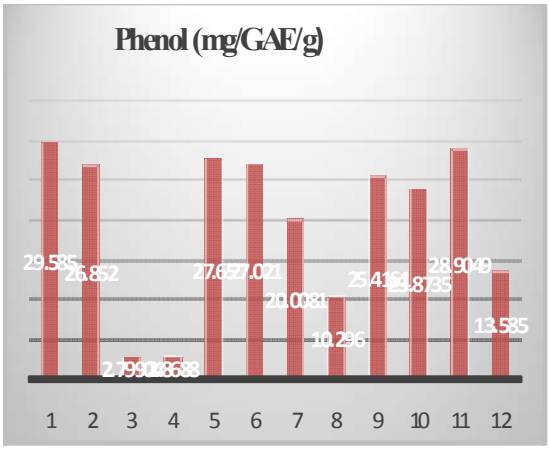

Fig-2: Graph representing Total phenolics of 12 different accessions of Fennel

Table 2. Total phenolic contents of different methanolic extracts of Fennel

Accession number	Plant Part	Solvent	Phenolic (mg/GAE /g of dry weight)
20575	Leaves	Methanol	29.585
20711	Leaves	Methanol	26.852
20786	Leaves	Methanol	26.799
20810	Leaves	Methanol	25.868
21396	Leaves	Methanol	27.65
21515	Leaves	Methanol	27.021
21539	Leaves	Methanol	20.00
21562	Leaves	Methanol	10.296
21757	Leaves	Methanol	25.41
21759	Leaves	Methanol	23.87
35858	Leaves	Methanol	28.90
38690	Leaves	Methanol	13.58

The highest flavonoids was observed in the accession number 38690.And the lowest flavonoids content was observed in accession number21515. On the other hand, the relationship between flavonoids and antioxidant activity was not observed. The result showed that the present study is in agreement with that of Miliauskas *et al.,*2004and Garcia-Alonso *et al.,*2004.

Table 3. Flavonoids content in different methanolic extract of Fennel

Accession number	Plant Part	Solvent	Flavonoids (CE/g DW)
20575	Leaves	Methanol	537.45
20711	Leaves	Methanol	438.125
20786	Leaves	Methanol	503.89

20810	Leaves	Methanol	570.45
21396	Leaves	Methanol	466.11
21515	Leaves	Methanol	386.65
21539	Leaves	Methanol	534.19
21562	Leaves	Methanol	106945
21757	Leaves	Methanol	457.1
21759	Leaves	Methanol	522.63
35858	Leaves	Methanol	415.28
38690	Leaves	Methanol	1526.45

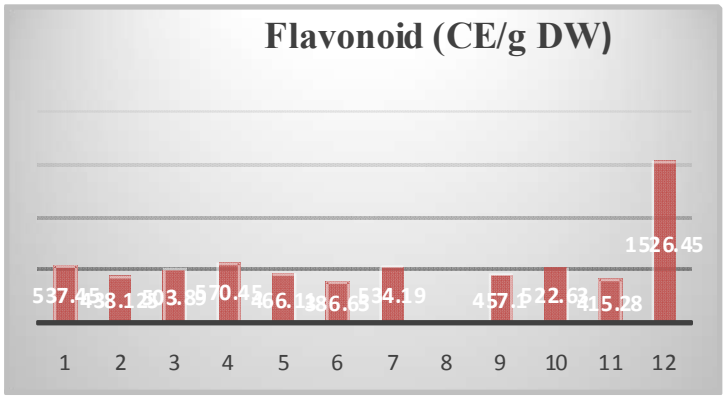

Fig-3: Graphic representation of flavonoids variation of Fennel (*Foeniculumvulgare).*

The highest antioxidant activity can be observed in methanolic extract of accession number 20575 which contains 45.42 %.The lowest antioxidant activity was observed in accession number 20786 which is 17.58%.Table 6 detailed total Polyphenols content of accessions of fennel.

Table 4. Antioxidant activities in different accessions

Accession number	Plant Part	Solvent	Antioxidant Potential % (DPPH)
20575	Leaves	Methanol	45.42%
20711	Leaves	Methanol	22.01%
20786	Leaves	Methanol	17.58%

20810	Leaves	Methanol	18.59%
21396	Leaves	Methanol	23.51%
21515	Leaves	Methanol	18.69%
21539	Leaves	Methanol	27.63%
21562	Leaves	Methanol	17.78%
21757	Leaves	Methanol	22.31%
21759	Leaves	Methanol	19.91%
35858	Leaves	Methanol	17.58%
38690	Leaves	Methanol	21.12%

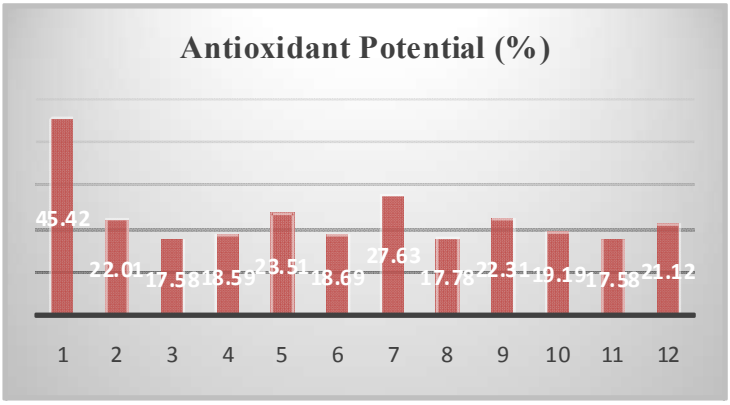

Fig-4: Variation in antioxidant activity in 12 accession of fennel

The study proved that there is enough variation in fennel for several yield-attributing traits. Such information would be important to indicate the effect of geographic origin on morphological and biochemical traits of fennel seed cultivars. Promising fennel cultivars can be used in various breeding programs and have the potential of enhancing its utilization.

The study found that there is enough variation in fennel for several yield-attributing traits, which can be used to advance this crop. Additionally, it is proposed that in order to increase fennel seed yield, greater attention should be paid to plant height, principal branches, and effective umbels. Promising fennel cultivars can be used in various breeding programs and have the potential of enhancing its utilization. The regular antioxidant diet includes fennel, which is considered as a great source of natural antioxidants (Shahat, 2011) **.** The aerial sections of the Italian fennel populations demonstrated the highest DPPH sca-venging activity, and wild fennel was shown to an

exhibit free radical scavenging activity with higher amount of phenolic and flavonoid than medicinal and edible fennel (Faudale, Viladomat, Bastia, Poli, Codina, 2008)**.** There were several fennel phenolic components that show antioxidant activity, including caffeoylquinic acid, rosmarinic acid, eriodictyol-7-orutinoside, quercetin-3-O-galactoside, and kaempferol-3-O-glucoside **(Parejo, 2004)**. Comparing the volatile oil to butylated hydroxyanisole and butylated hydroxytoluene, the volatile oil demonstrated high antioxidant activity. Fennel extracts in ethanol and water displayed lower antioxidant activity than the essential oil (Díaz-Maroto, 2005).

4. CONCLUSION

The main objective of this study was to determine the morphological and biochemical diversity of fennel seeds stored in a seed bank using morphological markers and to determine the variability in biochemical content among fennel plant accessions .12 accession were tested morphologically and biochemically. The results showed variation in height and number of branches, polyphenol and flavonoid content, and antioxidant potential among all accessions. Still there is a need for use of molecular markers and advanced research to study its genetic diversity. This study may help in effective management of crop germplasm and in improving the quality, quantity and breeding of fennel.

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