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Phytochemical mediated stimulation of ferulic acid using *Beta vulgaris* Linn. extract in *Foeniculum vulgare* Mill.

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Abstract
Phytochemicals are produced in plants through primary metabolites or secondary metabolites. They play a vital role in the lives of the plants. In this experiment seeds of *Foeniculum vulgare* Mill. Were germinated and when the plants increased in length, 4 sets were made, 1 set for control and 3 sets for giving the treatment of beet root extract (3.3%, 16.5% and 33%). After 21 days the plants were removed from the soil. Aqueous extract was prepared from the plants and phytochemical analysis was done. Phenols, tannins, terpenoids and flavonoids were found to be present. The aqueous extracts were used for studying thin layer chromatography. The Retention factor value for control set was observed to be 0 whereas the other sets showed the Retention factor value similar to that of ferulic acid.

Keywords: *Foeniculum vulgare* mill, *Beta vulgaris* Linn, ferulic acid, aqueous extract and thin layer chromatography

Introduction
The chemicals produced in plants through primary or secondary metabolites are known as Phytochemicals. They are natural bioactive nonreactive compound found in plants that protect and prevent diseases [2]. Some of the commonly occurring secondary metabolites are alkaloids, glycosides, polyphenols and terpenes. Phenols play a vital role in plants, they protect against ultraviolet radiation, pathogens, oxidative damage and harsh climatic conditions and contain pharmacological properties in them.
Among the phenols there is a phenol named Ferulic acid which is found in the plant cell wall polysaccharides. It has properties like anti-aging, anti-inflammatory etc. and is considered very useful in pharmacological industries.
Beta vulgaris Linn. Belonging to the family Chenopodiaceae is considered as one of the important tuber crops. It contains various polyphenols and among the phenols it contains ferulic acid in a very high concentration (800 mg).
Foeniculum vulgare Mill. Belonging to the family Apiaceae (Formerly Umbelliferae) is an important spice and is considered as a traditionally important medicine. The content of Ferulic acid is in a very low percentage (3.555%) which can be stimulated using phytochemical means.
The present study aims to stimulate the content of ferulic acid in *Foeniculum vulgare* mill. By using the extracts of *Beta vulgaris*.

Materials and Methods
Seeds from a local grocery store were bought and grown in small plastic glasses. The soil used to grow the seeds were taken from the Botanical garden of Gujarat University. Watering was done on regular basis. Seed germination occurred after 2 weeks of sowing. Once the height of the plants increased, 4 sets were made for the experiment, 1 set for control and 3 sets for giving the treatment of beet root extract. 1 gm, 5 gm and 10 gm of beet root was weighed and was crushed in the mixer grinder. The crushed beet roots were dissolved in distilled water to make the final volume 30ml. Thus, percent solutions were made for 1 gm (3.3%), 5 gm (16.5%) and 10 gm (33%). This was filtered using the filter paper (9cms) and the filtrate was poured in the soil containing plants. The treatment was given for 21 days with an interval of 7 days. In between the intervals, plants of each set was watered with 5-6 ml of distilled water. On the 7th day, 14th day and 21st day the height of the plant, number of fresh leaflets and number of dry leaflets were measured. The plants were taken off from the soil on the 21st day and thoroughly washed with distill water to remove soil particles.

Dry plants were finely powdered using motor pastel then in 10 ml of distill water it was dissolved and kept for 24 hours at 25 ℃ in a clean place. Filtration was done using Whatman filter paper (9cm). The filtrate obtained was used as extract after drying it at room temperature.

Phytochemical analysis was done using the standard protocol of [3]. Tests for alkaloids, phenols, tannins, saponins and flavonoids were studied.

The extracts of the plants were used for thin layer chromatography using the standard protocol [4, 8]. Spray reagent 1% Ferric chloride was used to visualize the bands for ferulic acid.

Result

The seeds were grown in well fertilized soil, when the saplings increased in length 1 set was kept for control and the other 3 sets were given treatment, of 3.3%, 16.5% and 33% beetroot extract at the interval of 7 days. The growth rate was measured and the number of fresh and dried leaflets were noted. Based on the observed results remarkable changes were seen due to the treatment given to the saplings (Table-1). The control set showed increase of the plant length very slowly and there was not much difference in its growth length it just showed 2cm increase from the 0 to 21st day. In the treated set of 16.5%, the plantlet showed the highest growth rate. The number of fresh leaflets were highest in the 16.5% treated and lowest in the control set. The number of dry leaves highest in the control and the lowest in the set of 16.5% treated sets.

Phytochemical analysis: The qualitative screening of aqueous fennel plants was done for secondary metabolites alkaloids, phenols, terpenoids, tannins, saponin and flavonoids (Table-2). Alkaloids were found to be absent in all the four phytochemical tests (Dragendroff's, Hager, Mayer and Wagner test). For phenols two phytochemical tests were done (Dichromate and Lead acetate test). In which the lead acetate test showed the presence of phenols in all the extracts while dichromate test showed its absence for the control set. For terpenoids only one phytochemical test was performed (Lieberman Burchard's Test) in which terpenoids were present in all the sets. Tannins were found absent in the control set where as in the rest of the three treated sets it was present (Bromine water test, ferric chloride test and gelatin test). For saponins only one phytochemical test was performed (foam test) which showed its absence in all the sets. Flavonoids were tested using two phytochemical tests (lead acetate test and ferric chloride test), it was found present in all the three sets.

Thin layer chromatography: In the current study, after the beet root extracts of varying concentrations were added to the soil for observing the increase in the concentration of the ferulic acid, thin layer chromatography was performed to quantify its content. Thin layer chromatography of aqueous extract of fennel in the solvent system chloroform: methanol: formic acid (85:15:1), and spray with $FeCl_3$ aqueous solution showed the retention factor values ranging from 0 to 0.71 (Fig-1).

Discussion: The 16.5% beetroot extract treated plantlets showed highly positive result in comparison to the other 3 sets within the time period of 21days. The number of dry leaflets of the 16.5% beetroot extract treated were also in less number in comparison to the other sets. After the treatments on the 21st day the plantlets were taken out from the soil and their photographs were taken. Based on the reviewed research works on fennel growth there has been no research work done in reference to a plant extract used to enhance the quantity and quality of a major compound present in it (ferulic acid).

Phytochemical analysis: Based on the reviewed paper it can be observed that the study of fennel has been done by various authors and the aqueous extract was used by [1, 9]. The positive result for presence of phenols, terpenoids, tannins and flavonoids were reported [1, 9]. The presence of alkaloids and saponins have been reported to be present by other researchers whereas, both were found to be absent in the current study.

Thin layer chromatography: According to the work done by [4], the methanolic extract when left for separation in chloroform: methanol: formic acid (85:15:1) as the mobile phase showed the formation of bands after the adsorption. The developed plates had been dried in a steam of hot air along with 1% ferric chloride (spray reagent) and kept at 254 nm and 366nm (fluroscence blue colour, 0.48±0.02) under UV light for the band visualization. In the present study aqueous extracts of fennel was used wherein the solvent system and spray reagent was used according to [6] showing the retention factor values ranging from 0 to 0.71.

In the control set after the adsorption of the extract on the silica plate the spot did not run even though it was placed in the solvent system. The 3.3% treated set resulted in developing a pale yellow band at retention factor value 0.5 while similar color band was seen at an retention factor value 0.4 for 16.5%. The obtained coloured band for both the treated sets (3.3and 16.5%) were almost similar to that of p-coumaric acid the precursor of ferulic acid according to [4]. The 33% treated set resulted in developing brownish-yellow band at retention factor value 0.71, whose values were differing in comparison to the other treated sets (3.3 and 16.5%). According to [8] the retention factor value for ferulic acid was 0.70±0.01 in the same solvent system that contradicts with a nonpolar compound "anethole" (0.71) in fennel. On comparing the results of [7] and [5] retention factor value 0.74 was similar to a non-polar compound "anethole" (0.71) that differs from [8].

Table 1: Plant length, Fresh leaflets and Dry leaflets

Plant Length				
Day	**Control**	**3.3%**	**16.5%**	**33%**
0	16	13.5±1.76	14.53±1.69	13.27±2.66
7	17	14.67±1.33	16±2.31	14.67±2.40
14	17.8	16±1.53	20.17±1.92	17.33±0.88
21	18	23±2	23±4.04	20.33±2.33
Fresh Leaflets				
Day	**Control**	**3.3%**	**16.5%**	**33%**
0	2	2.33±0.33	2.33±0.33	1.33±0.58
7	3	3.33±0.67	3.33±0.33	3.33±0.58
14	3	4±0.58	5.66±1.2	4±1.73
21	0	4.33±0.33	4.66±1.2	2.66±2.52
Dry Leaflet				
Day	**Control**	**3.3%**	**16.5%**	**33%**
0	0	0	0	0
7	0	0	0	0
14	2	1±0	0.33±0.33	1±0.58
21	3	2±0.58	1.33±0.33	2±0.58

Table 2: Qualitative Screening of Fennel plantlets (Aqueous extracts)

Phytochemicals	Tests	D.W	3.3%	16.5%	33%
Alkaloid	Dragendroff's Test	-	-	-	-
	Hager's Test	-	-	-	-
	Mayer's Test	-	-	-	-
	Wagner's Test	-	-	-	-
Phenol	Dichromate Test	-	+	++	++
	Lead Acetate Test	+	++	+++	+++
Terpenoids	Lieberman Burchard's Test	+	++	+++	++
Tannins	Bromine Test	-	+	++	++
	Ferric Chloride Test	-	+	++	++
	Gelatine Test	-	+	++	++
Saponins	Foam Test	-	-	-	-
Flavonoid	Lead Acetate	+	+	++	++
	Ferric Chloride Test	+	+	++	++

(Where + = present and - = absent)

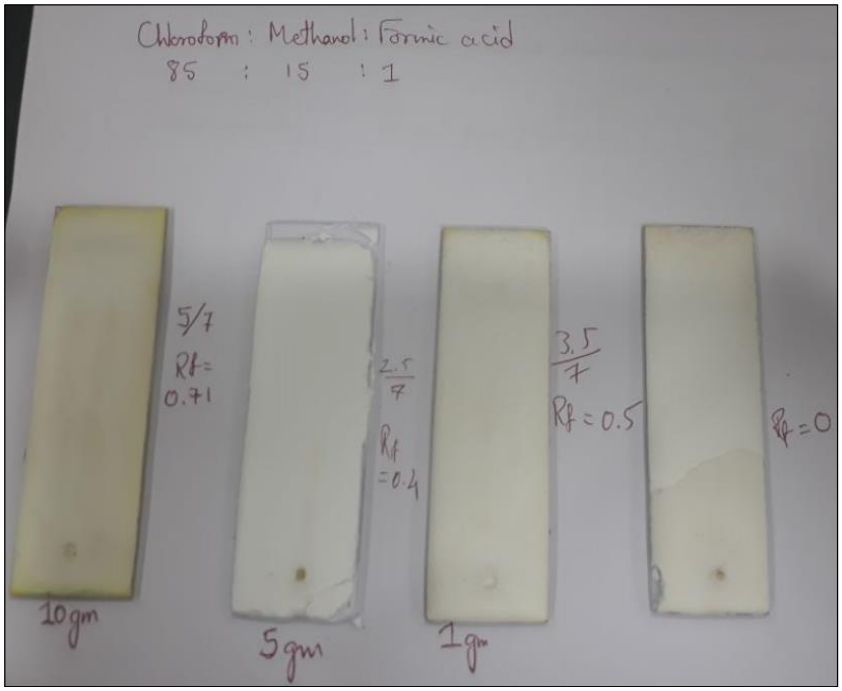

Fig 1: Thin layer chromatography for ferulic acid

Conclusion

Based on the results of the experiment performed, it can be assumed that the partially polar phenolic compound has been absorbed from the soil by the fennel plants as there has been remarkable changes observed in the plantlets of the treated set as compared to the control set. The physiological parameters and the phytochemical analysis has provided positive results in the treated sets in comparison to the control set.

The retention factor value shows that the beet root extract has enhanced the content of ferulic acid in the treated sets as the spot did not run at all in the control set.

As the higher concentration treated plant showed a varying range of retention factor value there is a probability that some changes may have occurred in the biosynthetic pathways of the fennel plant.

References

1. Beyazen A, Dessalegn E, Mamo W. Phytochemical screening and biological activities of leaf of *Foeniculum vulgare* (Ensilal). International Journal of Chemical Studies. 2017; 5(1):18-25.
2. Fernandes AJ, Gupta U, Sharma D, Mankad AU, Solanki HA. Antioxidant potentials in leaves of Kigelia, Thespesia, Eucalyptus, Peltophorum, and Azadirachta. International Journal of Advances in Pharmacy, Biology and Chemistry. 2015; 4(3):620-627.
3. Banu KS, Cathrine L. General Techniques Involved in Phytochemical Analysis. International Journal of Advanced Research in Chemical science. 2015; 2(4):25-32.
4. Mabinya LV, Mafunga T, Brand JM. Determination of ferulic acid and related compounds by thin layer chromatography. African Journal of Biotechnology. 2006; 5(13):1271-1273.
5. Bahreini M, Torabi S, Omidi M, Vazan S. Phytochemical study of Iranian Fennel (*Foeniculum vulgare*) Cell suspension Extract. Journal of Biology and Today's world. 2013; 2(11):498-500.
6. Najdoska M, Bogdanov J, Zdravkovski Z. TLC and GC-MS Analyses of Essential Oil Isolated from Macedonian Foeniculi fructus. Macedonian pharmaceutical bulletin. 2011; 56(1, 2):29-36.
7. Anubhuti P, Rahul S, Kant KC. Standardization of Fennel (*Foeniculum vulgare*), Its Oleoresin and Marketed Ayurvedic Dosage Forms. International Journal of Pharmaceutical Sciences and Drug Research. 2011; 3(3):265-269.

8. Tee-ngam P, Nunant N, Rattanarat P, Siangproh W, Chailapakul O. Simple and Rapid Determination of Ferulic Acid Levels in Food and Cosmetic Samples Using Paper-Based Platforms. Sensors. 2013; 13:13039-13053.
9. Chatterjee S, Goswami N, Bhatnagar P. Estimation of Phenolic Components and *in vitro* Antioxidant Activity of Fennel (*Foeniculum vulgare)* and Ajwain *(Trachyspermum ammi)* seeds. Advances in Bioresearch. 2012; 3(2):109-118.