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Phytochemical screening and *in vitro* antioxidant assays in *Foeniculum vulgare* Mill. (Fennel) seeds collected from Tarai region in the Uttarakhand

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Fennel is a culinary spice of Apiaceae family. The objective of present study to evaluate the antioxidant potential and phytochemical screening in terms of phenolic content, flavonoid content, orthodihydric phenolic content, proanthocyanidin, and total tannin content in hexane, chloroform, and methanol extract of *Foeniculum vulgare* seeds. Maximum DPPH scavenging activity exhibited in the hexane extract of FNL-PM (IC_{50}= 443.88±0.91 µg/mL). Maximum hydroxyl radical scavenging activity observed in methanol extract of FNL-117 (IC_{50}= 414.74±1.35 µg/mL). Higher superoxide radical scavenging activity exhibited in the hexane extract of FNL-126 (IC_{50}= 420.68±1.09 µg/mL) and nitric oxide radical scavenging in the hexane extract of FNL-117 (IC_{50}= 557.03±0.86 µg/mL). Strong metal chelation activity observed in hexane extract of FNL-118 (IC_{50}= 563.07±0.07 µg/mL). Higher phytochemicals are observed in methanol extract of FNL-120. The present study also revealed the similarities that exist between fennel seeds on the basis of antioxidant potency and phytochemicals through a statistical approach i.e., cluster analysis using dendrogram, regarding genetic makeup of fennel cultivars so that future research will be carried out to develop a new variety of fennel for further implementation in pharma industries.

Keywords: Antioxidants, DPPH scavenging, Fennel, Metal chelation scavenging, Phytochemicals, Superoxide scavenging.

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Introduction

Medicinal plants also called therapeutic herbs, have been discovered and used in traditional medicinal methods. Due to numerous favourable implementations, medicinal herbs and spices remain strongly in demand, in the functional food and biopharmaceutical industries[1]. Medicinal herbs are good alternative to chemical drugs, as they have no side effects than the synthetic drugs[2]. Medicinal herbs and spices are commonly used internally as decoction[3]. Spices and herbs are the most valuable targets to find the natural antioxidants and antibacterials[4,5]. The antioxidant property is mainly due to the redox action of the phenolic constituents[6]. Natural compounds from plant are emphasizing due to their potential used in pharmaceuticals and feasibly as natural herbicides[7]. Natural antioxidants are most effectively used to reduce the intensity of antioxidants[8]. Medicinal herbs and spices have high phenolic levels and demonstrated the higher antioxidant capacity[9]. Herbs and spices are among the most important targets for natural antioxidants from a health and protection point of view[10]. Photochemical and other chemical constituents of medicinal plants are known for their medicinal value[11]. Phenolic compounds commonly exist in bound forms in the plant species[12]. Medicinal plants are potent source of antioxidants and presence of photochemical in medicinal plants represents a number of pharmacological actions[13]. Due to consumer choices, the food industries are inclined to use natural antioxidants obtained from plant material[14]. Lipid oxidation adversely affects on flavour, nutritional value and overall quality of food. Fennel is a culinary spice of Apiaceae family mainly cultivated in world's tropical and temperate areas and has pharmacological activities like anti-inflammatory, antitumor, antimutagenic, cardiovascular activity[15]. The antioxidant activity of fennel seed extract was evaluated by synthetic antioxidant[16]. Phenolic compounds have earned considerable attention from medical researchers and nutritionists. Phenolic components present in fennel are known to be associated with the prevention of oxidative stress-induced diseases, such as cardiovascular disorders, cancer and inflammation[17]. A broad range of bioactive components are present in fennel as well as polyphenols having inferior bioaccesibility during gastrointestinal digestion[18]. Fennel is used for various purposes in the food industry, cosmetics, and

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medication[19]. The purpose of this study was to evaluate the phytochemical and antioxidant potential in hexane, chloroform, and methanol extract of fennel seeds collected from Tarai region in the Uttarakhand.

Materials and Methods

Source of plant material

Different varieties of fennel seed were collected from Vegetable Research Centre (VRC) Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, Pantnagar, Uttarakhand India for the experimental analysis. These varieties of fennel were developed in Pantnagar Terai region lies at the Latitude of 290 N, Longitude of 79.380 E and an Altitude of 243.84 MSL viz. FNL-116, FNL-117, FNL-118, FNL-119, FNL-120, FNL-121, FNL-123, FNL-125 and FNL-126 & FNL-PM.

Preparation of seed extracts

Fennel seeds were shade dried in laboratory and grounded to fine powder using a mixture grinder. Precisely 10 g of powder sample of fennel was taken and mixed in the ratio 1:5 with 50 mL of hexane, chloroform, and methanol solvent, respectively and left for 72 h with occasional stirring. The mixture was then centrifuged at 4000 rpm for 10 min. The supernatant was collected carefully and concentrated by using a vacuum rotatory evaporator at 40°C and stored in a deep freezer for further analysis.

Phytochemical analysis

Phytochemical analysis in hexane, chloroform, and methanol extracts of fennel seeds were done in terms of total phenolic content, total flavonoid content, orthodihydric content, proanthocyanidins content, and total tannins content with the help of their respective calibration curve obtained calorimetrically.

Total phenolic content

The total phenolic (TPC) content in hexane, choloroform, and methanolic extracts of fennel seeds was estimated by the method of Singleton and Rossi[20] with slight modification by Chandrasekara and Shahidi[21]. Exactly 0.5 mL of fennel seed extract was mixed with 0.5 mL Folin Ciocalteu Reagent reagent in a centrifuge tube. To neutralize the reaction, 1 mL of saturated Na_2CO_3 solution was added in each test tube and made up to 10 mL with distilled water for the final volume. The mixture was incubated in the centrifuge tubes in dark at 24°C for 35 min. Further, the mixture was centrifuged at 4000 rpm for 10 min and the absorbance was taken at 725 nm against reagent blank. The TPC was determined by establishing a standard curve using different concentrations of gallic acid and results were recorded as mg gallic acid equivalents (mg $GAEg^{-1}$).

Total flavonoid content

The total flavonoid content (TFC) was determined by the method Kim *et al.*[22] with some modification by Chandrasekara and Shahidi[23]. Mixed 1 mL of fennel extract with 4 mL of distilled water. To which 300 µL of $NaNO_2$ was added in the centrifuge tubes and allowed to incubate for 5 min. To the reaction mixture, 300 µL of $AlCl_3$ was added and allowed to stand for 1 min followed by the addition of 2 mL of 1M NaOH and 2.4 mL distilled water. After instant mixing, these solutions were incubated in dark for 15 min at 24°C. The mixture was centrifuged at 4000 rpm for 5 min and the absorbance was recorded at 510 nm. The TFC was reported as mg catechin equivalents (mg $CAEg^{-1}$).

Orthodihydric phenol content

The estimation of orthodihydric phenol was carried out by using method of Mahadevan and Shridhar[24]. Exactly1 mL of extract was taken in each test tube and added 1 mL of HCl followed by 1 mL Arnow's reagent (10 g of sodium nitrite and sodium molybdate, each dissolved in 100 mL distilled water). To the above mixture, 2 mL of NaOH solution was added and diluted with 4.5 mL of distilled water. Solutions were mixed properly and absorbance was read at 515 nm against reagent blank. The amount of orthodihydric phenol was determined from the standard curve using Catechol (10-120 µg) and amount was reported as mg catechol equivalents (mg/CLE/g).

Proanthocyanidin content

The amount of proanthocyanidins in the fennel extracts was evaluated by the method of Sun *et al.*[25]. Exactly 1 mL of fennel extract was taken and mixed with 3 mL of vanillin solution followed by the addition of 1 mL concentrated HCl. Each mixture was incubated in the dark for 15 min and the absorbance was recorded at 500 nm. Standard curve was expressed as mg catechin equivalents (mg $GAEg^{-1}$).

Total tannins

Total tannins were estimated by the method of Sadasivam and Manickam[26]. Exactly 1 mL of extract was taken and mixed with 6.6 mL of water followed by the addition of 0.5 mL of Folin-Denis reagent, 1 mL of Na_2CO_3 solution and made up to the 10 mL of volume with distilled water. The test mixture was

allowed to incubation for 30 min at room temperature and the absorbance was recorded at 700 nm against reagent blank. The amount of tannin was calculated from the standard curve using tannic acid (10-100 µg) as standard was reported as mg tannic acid equivalents (mg/TAE/g).

Antioxidant activity

In the present study, antioxidant activity was evaluated by DPPH radical scavenging method, hydroxyl radical scavenging, nitric oxide scavenging, superoxide radical scavenging, and metal chelation activity as compared to standard antioxidant.

DPPH radical scavenging activity

DPPH radical scavenging in hexane, chloroform and methanolic extracts was determined by the method given by Chen and Ho[27]. Exactly 1 mL of five different concentrations of extracts (200-1000µg/mL) was taken. To which 5 mL of DPPH solution was added and then, reaction mixture was kept in the dark at room temperature for 30 min. The absorbance of mixture was recorded at 517 nm against blank. The control and standard were subjected to the same procedure. Ascorbic acid and Gallic acid was used as standard. DPPH radical scavenging activity was calculated in the terms of % inhibition.

$$\% \text{ inhibition} = [(A-B)/A] \times 100$$

where, A = Absorbance of control and B = Absorbance of sample.

DPPH scavenging potential was discussed in terms of IC_{50}.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging was determined by the method described by Olabiniri *et al.*[28]. To a reaction mixture containing 60 µL of $FeSO_4.7H_2O$, 90 µL of 1 mL 1,10- Phenanthroline, 2.4 mL of 0.2 M phosphate buffer (pH 7.8), and 1 mL of five different concentration of extract (200-1000 µg/mL) was added. Exactly 150 µL of hydrogen peroxide was added in each sample to start the reaction. After 5 min of incubation at room temperature, the absorbance was measured at 536 nm. Ascorbic acid was taken as standard. Hydroxyl radical scavenging activity was calculated in terms of % inhibition.

$$\% \text{ inhibition} = [(A-B)/A] \times 100$$

where, A = Absorbance of control and B = Absorbance of sample.

Hydroxyl scavenging activity was discussed in terms of IC_{50}.

Nitric oxide radical scavenging activity

The nitric oxide radical scavenging in was determined by Naskar *et al.*[29]. Exactly 2 mL of sodium nitroprusside (10 mM) was taken and dissolved in phosphate buffer saline (pH 7.4) and mixed with five different concentrations of extract (200-1000 µg/mL). The reaction mixture was incubated for 2.5 h at 25 °C followed by the addition of 1 mL Griess reagent. The absorbance was measured at 536 nm. Ascorbic acid was taken as standard. Nitric oxide radical scavenging activity was calculated by % inhibition.

$$\% \text{ inhibition} = [(A-B)/A] \times 100$$

where, A = Absorbance of control and B = Absorbance of sample.

Nitric oxide scavenging potential was discussed in terms of IC_{50}.

Superoxide radical scavenging activity

The method of Nishimiki *et al.*[30] was adopted for the estimation of superoxide radical scavanging activity in the fennel seed extracts with slight modifications. This method is based on principal of reduction of nitrobluetetrazolium. The reaction mixture of 1 mL of nitrobluetetrazolium (NBT) solution and 1 mL of NADH soliution was mixed with 0.1 mL of five different concentrations of hexane chloroform and methanol extracts (200-1000 µg/mL). The reaction was started by the addition of 100 mL of Phenazine methosulphate (PMS) solution to all mixtures and incubated for 5 min at 25 °C. The absorbance at 560 nm was measured against blank samples, containing all the reagents except the PMS. Ascorbic acid was used as standard. Superoxide radical scavenging was calculated by % inhibition.

$$\% \text{ inhibition} = [(A-B)/A] \times 100$$

where, A = Absorbance of control and B = Absorbance of sample.

Superoxide radical scavenging potential was discussed in terms of IC_{50}.

Metal chelation activity

Metal chelation activity in the fennel extracts was determined by using the method followed by Hsu *et al.*[31]. This method is based on principle of Fe^{2+} chelation ability of the antioxidants by measuring the ferrous iron-ferrozine complex formed at 562 nm. Five different concentrations of the extract (200-1000 µg/mL) were mixed with 0.1 mL of 2 mM $FeCl_2.4H_2O$ and 0.2 mL of 5 mM ferrozine. The final volume of mixture was made to 5 mL with 4.7 mL methanol and

allowed to react for 10 min. EDTA was used as standard. The absorbance was measured at 562 nm. Metal chelation was calculated by % Chelation.

$$\% \text{ Chelation} = [(A-B)/A] \times 100$$

where, A = Absorbance of control and B = Absorbance of sample.

Metal chelation potential was discussed in terms of IC_{50}.

Statistical analysis

All the experiments were conducted in triplicates and results were analyzed as Mean±SD (standard deviation). Correlation study and statistical analysis were done using SPSS 20 software (Statistical Package for Social Science). Cluster analysis was also performed using SPSS 20 software by applying Ward's method with Squared Euclidean distance.

Results and Discussion

A noticeable variation was observed in percentage yield of hexane, chloroform and methanol extract of fennel seeds as presented in Table 1. The % yield in the fennel seed extract was found to be between 3.52 to 10.05%. However, in a previous report[32], the per cent yield of the fennel seed extract was observed to range from 6.21 to 15.63%. In the present study, the yield in hexane extract (10.05%) of the fennel seeds was comparatively higher followed by chloroform (9.75%) and methanol extract (8.75%). This suggests that fennel seeds contain higher soluble non-polar components.

The phytochemical screening of the hexane, chloroform, and methanol extract of the fennel seeds is presented in Table 2. The TPC in the hexane extract ranged between 1.43±0.40 to 7.10±0.51 mg $GAEg^{-1}$, in chloroform extract between 1.34±0.39 to 0.76±0.01 mg $GAEg^{-1}$ and in methanol extract between 4.78±1.56 to 32.90±0.79 mg $GAEg^{-1}$. The TFC in the hexane extract of fennel seeds ranged between 0.57±0.26 to 2.98±0.65 mg $CAEg^{-1}$, chloroform extract between 0.40±0.19 to 1.95±0.35 mg $CAEg^{-1}$ and methanol extract between 0.57±0.13 to 11.92±1.86 mg $CAEg^{-1}$.

Table 1 — Percent yield in hexane, chloroform and methanol extract of fennel seeds

S. No.	Fennel seeds	% Yield		
		Hexane extract	Chloroform extract	Methanol extract
1	FNL-116	10.05	7.50	4.45
2	FNL-117	8.35	6.00	4.00
3	FNL-118	5.05	5.15	3.95
4	FNL-119	4.95	9.10	3.52
5	FNL-120	7.45	9.20	4.10
6	FNL-121	3.70	9.75	4.25
7	FNL-123	3.90	5.25	4.05
8	FNL-124	5.70	5.65	3.65
9	FNL-125	5.25	5.45	4.10
10	FNL-126	5.25	5.10	4.60
11	FNL-PM	9.50	6.25	8.75

The orthodihydric phenol amount was observed to be in the range of 1.36±0.14 **to** 31.75±0.08 mg/ CLE/g in the hexane extract, 1.34±0.35 to 49.41±4.3 mg/ CLE/g in chloroform extract, and 22.46±0.26 to 59.57±0.30 mg/CLE/g in methanol extract of the fennel seeds. The proanthocyanidins content was found to be in the range of 0.55±0.24 to 15.59±0.28 mg $CAEg^{-1}$ in the hexane extract, 0.88±0.55 - 22.42±0.21 mg $CAEg^{-1}$ in the chloroform extract, and 10.47±0.17 - 33.62±0.34 mg $CAEg^{-1}$ in the methanol extract of fennel seeds.

The total tannins content was observed to be in the range of 3.01±0.10 to 5.52±0.07 mg/TAE/g in the hexane extract, 1.03±0.03 to 7.47±0.32 mg/TAE/g in the chloroform extract, and 3.67±0.22 to 8.42±0.32 mg/TAE/g in the methanol extract of fennel seeds.

Phytochemicals varied among the different solvent systems. Polar and mid polar solvents like methanol and chloroform have best phytochemical potential than non-polar solvent. A previous report found that fennel seed extracts contained significant levels of TPC (627.21–967.50 GAE, mg/100 g) and TFC (374.88–681.96 CE, mg/100 g)[33]. The TPC of methanol extract evaluated by using Folin Ciocalteu reagent was found to be 3.48±4.2 mg GAE/g of dried extract[34]. Phenolic content was higher in the methanol extract of fennel (21.6±0.32 mg $GAEg^{-1}$)[35]. In the present study, higher phenol content was observed in the methanol extract of FNL-117 (32.90±0.79 mg $GAEg^{-1}$) and higher flavonoid content was observed in the methanol extract of FNL-120 (11.92±1.86 mg $CAEg^{-1}$). The orthodihydric phenolic content was higher in the methanol extract of FNL-120 (59.57±0.30 mg/CLE/g). FNL-117 contained higher proanthocyanidins content (33.62±0.34 mg $CAEg^{-1}$). The total tannin content is higher in FNL-121 (8.42±0.32 mg/ TAE/g).

Antioxidant potential in hexane, chloroform, and methanol extract is given in Table 3. DPPH is used to check the ability of compounds to act as free radical scavengers and evaluate the antioxidant activity[36]. DPPH radical scavenging is an easy and potent method

Table 2 — Phytochemical screening in hexane, chloroform and methanol extracts of fennel seeds

Fennel seeds	Extract	Total phenolic content (mg GAEg^{-1})	Total flavanoid content (mg CAEg^{-1})	Orthodyhydric phenol content (mg/ CLE/g)	Proanthocyanidine Content (mg CAEg^{-1})	Total Tannins (mg/ TAE/g)
FNL-116	Hexane	1.34±0.39^{e}	2.35±0.21^{c}	1.52±0.21^{a}	0.55±0.24^{a}	4.91±0.24^{bc}
	Chloroform	1.34±0.39^{b}	0.97±0.26^{abcd}	1.52±0.21^{e}	19.69±0.13^{e}	2.99±0.13^{d}
	Methanol	6.87±0.74^{a}	8.96±0.34^{cd}	43.75±0.24^{g}	10.47±0.17^{a}	6.62±0.07^{d}
FNL-117	Hexane	0.87±0.02^{bcd}	0.97±0.64^{ab}	1.36±0.14^{a}	4.44±0.26^{b}	4.51±0.34^{b}
	Chloroform	0.87±0.02^{a}	0.97±0.43^{abcd}	1.36±0.14^{ab}	17.43±0.21^{d}	2.63±0.17^{cd}
	Methanol	32.90±0.79^{f}	0.57±0.13^{a}	8.54±0.48^{e}	33.62±0.34^{g}	3.67±0.22^{a}
FNL-118	Hexane	0.76±0.01^{abc}	1.83±0.49^{abc}	1.82±0.08^{a}	7.47±0.25^{d}	4.50±0.28^{b}
	Chloroform	0.76±0.01^{a}	1.39±0.89^{abcd}	1.82±0.08^{e}	22.42±0.21^{g}	2.2±0.10^{bc}
	Methanol	28.21±0.79^{e}	9.91±0.25^{cd}	41.13±4.52^{f}	26.50±0.43^{e}	4.77±0.13^{b}
FNL-119	Hexane	0.96±0.01^{abcd}	0.57±0.26^{a}	6.58±0.17^{c}	5.30±0.25^{bc}	3.01±0.10^{a}
	Chloroform	0.96±0.01^{a}	0.45±0.26^{ab}	6.58±0.17^{cd}	15.52±0.39^{c}	3.67±0.22^{e}
	Methanol	26.95±0.21^{e}	8.50±0.69^{bcd}	25.10±3.17^{d}	25.54±0.07^{de}	6.47±0.22^{d}
FNL-120	Hexane	0.91±0.01^{cd}	1.95±0.34^{bc}	28.50±0.23f	4.76±1.80^{bc}	4.60±0.225^{b}
	Chloroform	0.91±0.01^{a}	1.95±0.35^{d}	28.50±0.23^{d}	21.59±0.28^{f}	1.03±0.03^{a}
	Methanol	18.14±0.35^{c}	10.45±1.48^{cd}	29.55±7.93^{i}	27.15±0.65^{e}	5.00±0.10^{b}
FNL-121	Hexane	0.77±0.01^{e}	0.57±0.43^{a}	5.62±0.18^{b}	7.56±0.12^{d}	4.42±0.14^{b}
	Chloroform	0.77±0.01^{a}	0.80±0.26^{abcd}	5.62±0.18^{ab}	1.42±0.35^{ab}	7.47±0.32^{h}
	Methanol	11.93±0.94^{b}	7.35±1.50^{bc}	7.13±1.35^{a}	22.23±0.46^{c}	8.42±0.26^{e}
FNL-123	Hexane	1.05±0.03^{f}	2.98±0.65^{ab}	27.93±0.12^{f}	15.59±0.28^{g}	4.52±0.18^{b}
	Chloroform	1.05±0.03^{ab}	1.86±0.73^{cd}	27.93±0.12^{e}	21.68±0.17^{f}	1.83±0.11^{b}
	Methanol	7.83±1.08^{a}	10.34±1.36^{cd}	49.41±4.31^{e}	29.28±0.47^{f}	4.46±0.07^{b}
FNL-124	Hexane	0.87±0.02^{ab}	1.37±0.45^{abc}	13.96±0.14^{e}	9.61±0.18^{e}	4.84±0.35^{bc}
	Chloroform	0.87±0.02^{a}	0.60±0.31^{abc}	13.96±0.14^{ab}	1.69±0.12^{b}	2.82±0.09^{d}
	Methanol	4.78±1.56^{a}	5.02±1.68^{b}	7.18±0.9^{a}	15.72±0.27^{b}	6.54±0.31^{d}
FNL-125	Hexane	0.93±0.02^{a}	1.63±0.31^{abc}	31.75±0.08^{g}	6..48±0.36^{cd}	5.52±0.075^{c}
	Chloroform	0.93±0.02^{a}	1.72±0.17^{bcd}	31.75±0.08^{e}	1.56±0.22^{ab}	2.47±0.28^{cd}
	Methanol	11.89±1.51^{b}	11.92±1.86^{e}	41.49±7.09^{f}	10.58±0.32^{a}	6.75±0.21^{d}
FNL-126	Hexane	0.94±0.03^{bcd}	1.09±0.86^{abc}	5.25±0.91^{b}	10.69±0.15^{ef}	4.31±0.24^{b}
	Chloroform	0.94±0.03^{a}	0.40±0.19^{a}	5.25±0.91^{a}	0.88±0.055^{a}	5.35±0.31^{g}
	Methanol	14.95±1.04^{b}	10.77±0.90^{cd}	1.34±0.35^{c}	24.53±1.53^{d}	6.55±0.30^{d}
FNL-PM	Hexane	1.00±0.01^{de}	1.83±0.35^{abc}	7.58±0.12^{d}	11.54±0.33^{f}	3.47±0.29^{a}
	Chloroform	1.00±0.01^{ab}	1.03±0.34^{abcd}	7.58±0.12^{bc}	15.61±0.34^{c}	4.7±0.21^{f}
	Methanol	22.70±1.50^{d}	7.41±1.20^{bc}	14.91±2.43^{b}	21.97±0.61^{c}	5.79±0.16^{c}

Values are mean of three replicates±SD. Within a column, mean values followed by same alphabetic letter are not significantly different according to Tukey's test (P <0.05)

to evaluate the antioxidant potential in plant sample extract[37]. IC_{50} value of DPPH radical scavenging activity in hexane extract of fennel seeds was found to be 443.88±0.91 to 897.0±0.88 µg/mL. IC_{50} values in the hexane extract was observed to be in the order of FNL-PM> FNL-116> FNL-126> FNL-118> FNL-119> FNL-121> FNL-120> FNL-117> FNL-125> FNL-124> FNL-123. In the chloroform extract, IC_{50} value was found to be in the range of 633.80±1.07 to 1196.79±0.61 µg/mL. It was observed to be in the order of FNL-PM> FNL-116> FNL-117> FNL-118> FNL-121> FNL-120> FNL-119> FNL-125> FNL-123> FNL-126> FNL-124. In the methanol extract, IC_{50} value was observed to be in the range of 463.33±0.42 to 1099.36±0.45 µg/ mL. It was observed to be in the order of FNL-PM> FNL-126> FNL-119> FNL-125> FNL-121> FNL-120> FNL-124> FNL-123> FNL-116> FNL-118> FNL-117. In a previous study[22], the fennel seeds extract exhibited good DPPH scavenging activity, with IC_{50} values ranging from 23.61 to 26.75 µg/mL. Egypt and China fennel seed extract exhibited a superior DPPH radical scavenging activity with IC_{50} value as 6.34 and 7.17 mg/g, respectively[25]. Present results show that the maximum DPPH scavenging activity was exhibited by the hexane extract of FNL-PM (IC_{50} = 443.88±0.91 µg/mL).

In the terms of hydroxyl radical scavenging activity, IC_{50} value of the hexane extract of the fennel

Table 3 — Antioxidant activity in hexane, chloroform and methanol extracts of fennel seeds

S. No.	Fennel seeds	Extract	DPPH IC_{50} value (µg/mL)	Hydroxyl scavenging IC_{50} value (µg/mL)	Superoxide scavenging IC_{50} value (µg/mL)	Nitric oxide scavenging IC_{50} value (µg/mL)	Chelation scavenging IC_{50} value (µg/mL)
1	FNL-116	Hexane	546.10±0.77^{d}	438.11±0.63^{d}	497.11±0.947^{i}	672.19±0.92^{e}	799.16±0.42^{g}
		Chloroform	771.05±1.12^{d}	481.55±0.88^{c}	872.05±0.84^{g}	1202.64±0.65^{i}	569.02±0.76^{b}
		Methanol	909.66±0.67^{j}	452.08±0.77^{f}	915.04±0.97^{j}	1405.19±0.69^{h}	964.85±1.28^{l}
2	FNL-117	Hexane	807.53±1.09^{h}	431.76±1.27^{c}	425.04±1.16^{c}	557.03±0.86^{b}	826.97±0.53^{i}
		Chloroform	823.62±1.11^{e}	458.15±0.99^{b}	878.08±0.91^{h}	1073.25±0.47^{e}	679.14±0.69^{e}
		Methanol	1099.36±0.45^{k}	414.74±1.35^{b}	520.24±0.93^{f}	1313.84±1.37^{d}	827.10±1.30^{k}
3	FNL-118	Hexane	639.52±0.97^{e}	432.01±0.90^{c}	464.90±0.97^{f}	658.69±1.47^{d}	563.07±0.98^{b}
		Chloroform	839.37±0.40^{f}	503.03±0.99^{e}	848.75±1.18^{f}	1086.91±0.10^{g}	680.31±0.93^{e}
		Methanol	1139.41±0.43^{l}	436.83±1.17^{c}	483.85±1.16^{b}	1392.75±0.25^{g}	794.83±0.27^{i}
4	FNL-119	Hexane	663.91±1.46^{f}	426.19±0.90^{b}	539.21±0.899^{k}	678.98±0.56^{f}	1012.73±0.69^{k}
		Chloroform	1113.69±0.75^{i}	606.92±0.75^{j}	905.28±1.05^{j}	995.09±0.99^{b}	564.79±0.44^{b}
		Methanol	573.49±1.46^{e}	568.15±0.70^{k}	612.152±0.82^{i}	1563.13±0.67^{i}	754.81±1.63^{g}
5	FNL-120	Hexane	789.88±1.07^{g}	439.06±0.45^{d}	443.66±1.05^{d}	817.41±0.52^{j}	811.50±1.07^{h}
		Chloroform	1076.82±1.36^{h}	553.32±0.77^{h}	816.28±0.56^{d}	1078.33±0.82^{f}	755.91±0.54^{g}
		Methanol	607.15±0.96^{g}	446.43±0.37^{e}	538.98±0.72^{g}	1397±0.62^{g}	741.13±1.43^{cd}
6	FNL-121	Hexane	667.02±1.11^{f}	455.31±0.83^{f}	481.43±0.63^{g}	641.50±0.49^{c}	714.81±1.40^{e}
		Chloroform	1014.89±0.66^{g}	562.74±0.29^{i}	904.01±0.13^{i}	1273.05±0.72^{l}	854.23±0.98^{i}
		Methanol	605.67±0.46^{g}	469.10±0.89^{g}	520.20±0.94^{f}	1573.67±1.17^{j}	746.58±1.48^{de}
7	FNL-123	Hexane	897.01±0.88^{j}	463.25±0.32^{g}	459.17±0.48^{e}	658.29±0.51^{d}	629.90±0.48^{c}
		Chloroform	1166.33±0.76^{j}	517.03±0.96^{f}	815.11±0.73^{d}	1213.98±0.34^{j}	818.45±1.01^{h}
		Methanol	757.37±0.89^{i}	468.32±0.77^{g}	517.82±0.83^{f}	1313.91±1.45^{d}	769.98±1.01^{h}
8	FNL-124	Hexane	818.13±1.00^{i}	463.25±0.32^{g}	514.12±1.24^{j}	712.21±0.87^{i}	773.79±12.06^{f}
		Chloroform	1196.79±0.61^{l}	517.03±0.96^{f}	792.04±0.86^{c}	1040.23±1.02^{d}	739.28±0.65^{f}
		Methanol	641.13±0.83^{h}	468.32±0.77^{i}	499.13±0.59^{e}	1360.83±5.99^{e}	801.83±3.0^{j}
9	FNL-125	Hexane	810.62±1.36^{h}	454.83±1.36^{f}	457.53±0.46^{e}	704.10±0.87^{h}	649.77±0.39^{d}
		Chloroform	1116.52±0.46^{i}	534.36±1.77^{g}	752.08±1.20^{b}	1224.30±1.58^{k}	869.15±1.07^{j}
		Methanol	594.17±0.93^{f}	523.72±1.14^{j}	494.61±1.40^{d}	1219.15±0.65^{c}	731.54±0.50^{b}
10	FNL-126	Hexane	639.52±0.97^{e}	451.15±0.67^{e}	420.68±1.09^{b}	678.28±0.72^{f}	722.51±1.02^{e}
		Chloroform	1180.21±0.86^{k}	505.96±0.88^{e}	837.15±0.93e	1109.15±0.47^{h}	661.96±5.36^{d}
		Methanol	542.63±0.42^{d}	498.84±0.26^{h}	490.79±0.67^{c}	1369.63±0.20^{f}	739.26±0.66^{c}
11	FNL-PM	Hexane	443.88±0.91^{c}	449.27±0.38^{e}	487.01±1.02^{h}	697.81±0.44^{g}	881.39±1.06^{j}
		Chloroform	633.80±1.07^{c}	495.42±0.414^{d}	916.93±1.07^{k}	1029.25±0.54^{c}	644.89±1.22^{c}
		Methanol	463.33±0.42^{c}	440.96±1.11^{d}	553.55±0.40^{h}	1147.04±0.27^{b}	747.91±1.51^{f}
Ascorbic acid*			395.83±0.71^{b}	388.57±2.16^{a}	397.94±1.34^{a}	393.07±3.48^{a}	393.78±4.69^{a}
Gallic acid*			384.05±2.91^{a}	-	-	-	-

*= standard antioxidants, "–" = not applicable. Values are mean of three replicates±SD. Within a column, mean value followed by same alphabetic letter are significantly not different according to Tukey's test (P <0.005)

seeds was in the range of 426.19±0.90 to 463.25±0.32 µg/mL. It was observed to be in the order of FNL-119> FNL-117> FNL-118> FNL-116> FNL-120> FNL-PM> FNL-126> FNL-125> FNL-121> FNL-124> FNL-123. IC_{50} value in the chloroform extract was observed to be in the range of 458.15±0.99 to 606.92±0.75 µg/mL. It was found to be in the order of FNL-117> FNL-116> FNL-PM> FNL-118> FNL-126> FNL-124> FNL-123> FNL-125> FNL-120> FNL-121> FNL-119. It was observed to range from 414.74±1.35 to 568.15±0.70 µg/mL. It was found to be in the order of FNL-117> FNL-118> FNL-PM> FNL-120> FNL-116> FNL-123> FNL-121> FNL-126> FNL-124> FNL-125> FNL-119. It has been reported[38] that at 240 µg/mL concentration, the methanol extract of fennel seeds demonstrated the maximum OH- scavenging potential of 71.61%. Present results demonstrated that maximum hydroxyl radical scavenging activity observed in methanol extract of FNL-117 (IC_{50}=414.74±1.35 µg/mL).

IC_{50} value of superoxide radical scavenging activity in the hexane extract was observed to range from 420.68±1.09 to 539.21±0.89 µg/mL. It was observed to be in the order of FNL-126> FNL-117> FNL-120> FNL-125> FNL-123> FNL-118> FNL-121> FNL-PM> FNL-116> FNL-124> FNL-119. In

the chloroform extract, IC_{50} value ranged from 752.08±1.20 to 916.93±1.07 µg/mL. It was observed to be in the order of FNL-125> FNL-124> FNL-123> FNL-120> FNL-126> FNL-118> FNL-117> FNL-116> FNL-121> FNL-119> FNL-PM. IC_{50} value in the methanol extract was found to be in the range of 483.85±1.16 to 915.04±0.97 µg/mL. It was observed to be in the order of FNL-118> FNL-126> FNL-125> FNL-124> FNL-123> FNL-117> FNL-121> FNL-120> FNL-PM> FNL-119> FNL-116.

IC_{50} value of the nitric oxide radical scavenging in hexane extract of fennel seeds was observed to range from 557.03±0.86 to 817.41±0.52 µg/mL. It was in the order of FNL-117> FNL-121> FNL-123> FNL-119> FNL-116> FNL-119> FNL-126> FNL-PM> FNL-125> FNL-124> FNL-120. The chloroform extract exhibited IC_{50} value in the range of 995.06±0.99 to 1273.05±0.72 µg/mL. It was in the order of FNL-119> FNL-PM> FNL-124> FNL-117> FNL-121> FNL-120> FNL-118> FNL-126> FNL-116> FNL-123> FNL-125. IC_{50} value in the methanol extract was observed to range from 1147.04±0.27 to 1573.67±1.17 µg/mL. It was in the order of FNL-PM> FNL-125> FNL-117> FNL-123> FNL-124> FNL-126> FNL-119> FNL-118> FNL-120> FNL-116> FNL-121.

In terms of metal chelation scavenging potential, IC_{50} value in hexane extract of fennel seeds was observed to be in the range of 563.07±0.07 to 1012.73±0.69 µg/mL. It is in the order of FNL-118> FNL-123> FNL-125> FNL-121> FNL-126> FNL-124> FNL-116> FNL-120> FNL-117> FNL-PM> FNL-119. IC_{50} value in the chloroform extract was found to be in the range of 564.79±0.44 to 869.15±1.07 µg/mL. It is in the order of FNL-119> FNL-116> FNL-126> FNL-PM> FNL-117> FNL-118> FNL-124> FNL-120> FNL-123> FNL-121> FNL-125. IC_{50} value in the methanol extract was found to be in the range of 731.54±0.50 to 964.85±1.28 µg/mL. It is observed to be in the order of FNL-125> FNL-126> FNL-120> FNL-PM> FNL-121> FNL-119> FNL-123> FNL-118> FNL-124> FNL-117> FNL-116. Higher antioxidant potential was observed in the methanol extract of FNL-117 which depicts that higher phenolic constituents are present in it.

Purpose of the correlation study was to examine the interrelation between biochemical parameter and antioxidant activity in the fennel seeds. As seen in Table 4, negative correlation (-1.47, -2.15, and -362) was observed between the TPC and IC_{50} values of antioxidant activity (DPPH, Hydroxyl, and superoxide), which indicates that IC_{50} decreases and antioxidant activity increases with the increase in the TPC. Negative correlation (-0.347, -0.018, and -0.278) was also observed between TFC and antioxidant activity (DPPH, Hydroxyl, and superoxide). A good positive correlation (0.531 and 0.635) was observed between the TPC and NO radical scavenging, and also between the TFC and NO radical scavenging activity. The positive correlation between orthodihydric phenol content and antioxidant activity means higher antioxidant potential of fennel. In addition, a superior positive correlation (0.531, 0.635, 0.639, 0.580, and 0.353) was noticed between NO radical scavenging activity and all the biochemical parameters.

Cluster analysis

Cluster analysis was done on the basis of the antioxidant activity and phytochemical potential to represent the similarities in fennel seeds grouped into three clusters, presented in their respective dendrograms in Fig. 1-3, each as follows:

Table 4 — A correlation study between biochemical parameter and antioxidant activity of fennel seeds

	Total phenolic	Total flavonoid	Orthodyhdric phenols	Proanthocyanidin	Tannins	IC_{50} DPPH radical scavenging assay	IC_{50} OH radical scavenging assay	IC_{50} Superoxide radical scavenging assay	IC_{50} Nitric oxide radical scavenging assay	IC_{50} metal chelation assay
Total phenolic	1	.602**	.497**	.652**	.344ns	-.147ns	-.215ns	-.362 *	.531**	.141ns
Total flavonoid		1	.613**	.496**	.540**	-.347 *	-0.018	-0.278	.635**	0.141
Orthodyhdric phenols			1	.631**	0.012	0.174	0.113	0.101	.639**	0.091
Proanthocyanidin				1	-0.032	-0.086	-0.014	-0.034	.580**	-0.138
Tannins					1	-.415 *	-0.026	-0.299	.353 *	0.062
IC_{50} DPPH radical scavenging assay						1	.347 *	.506**	0.143	0.001
IC_{50} OH radical scavenging assay							1	.585**	.377 *	-0.215
IC_{50} Superoxide radical scavenging assay								1	0.308	-0.129
IC_{50} Nitric oxide radical scavenging assay									1	0.145
IC_{50} metal chelation assay										1

*P <0.05, **P <0.01, ns= non significant (P >0.05)

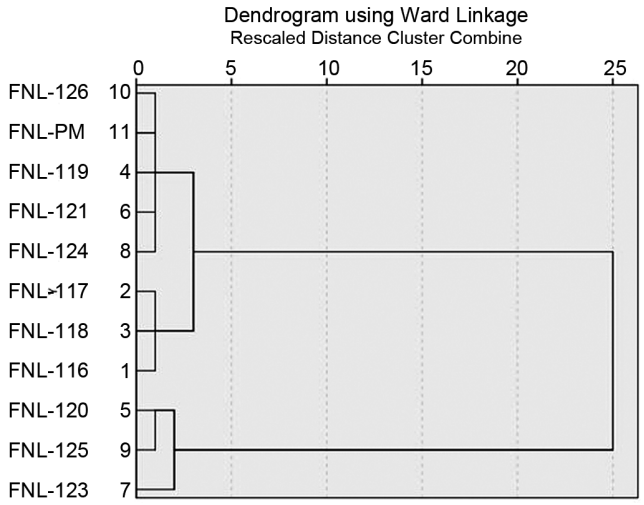

Fig. 1 — Cluster analysis in hexane extract of fennel seeds

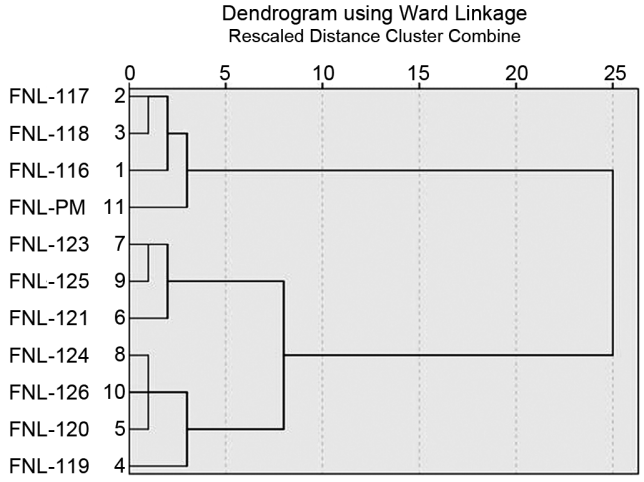

Fig. 2 — Cluster analysis in chloroform extract of fennel seeds

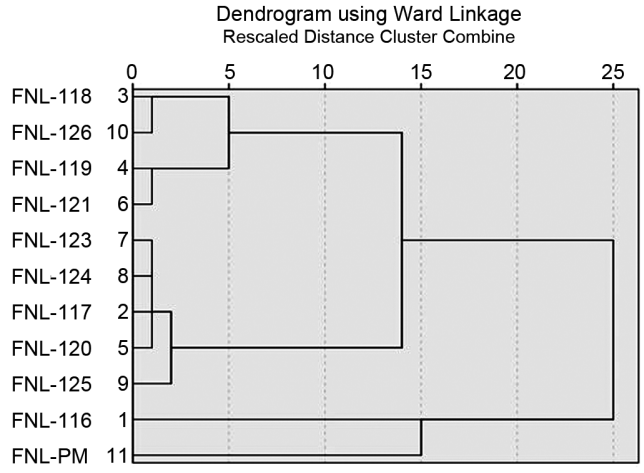

Fig. 3 — Cluster analysis in methanol extract of fennel seeds

Clustering in hexane extract

Group1: FNL-116, FNL-117, FNL-123
Group 2: FNL-119, FNL-121, FNL-124, FNL-126, FNL-PM
Group 3: FNL-120, FNL-123, FNL-125

Clustering in chloroform extract

Group1: FNL-116, FNL-117, FNL-118, FNL-PM
Group 2: FNL-119, FNL-120, FNL-124, FNL-126
Group 3: FNL-121, FNL-123, FNL-125

Clustering in methanol extract

Group1: FNL-116
Group 2: FNL-117, FNL-120, FNL-123, FNL-124, FNL-125
Group 3: FNL-118, FNL-119, FNL-121, FNL-126, FNL-PM

FNL-120, FNL-119, and FNL-126 represent in the similar cluster in hexane, chloroform, and methanol extract of the fennel seeds. In the present study, cluster analysis depicts that a new variety of fennel could be developed which contains rich phytoconstituents having a good antioxidant potential.

Conclusion

The present study concluded that hexane, chloroform, and methanol extracts of fennel seeds have a good phytochemical potential and have rich antioxidants. Due to the presence of an appreciable amount of phytoconstituents, the antioxidant potential of *Foeniculum vulgare* is highly effective and can be used as a good source of natural antioxidants. To the best of the authors' knowledge, this is the first report on phytochemical screening and antioxidant activity of fennel seeds developed and collected from Tarai region in the Uttarakhand state (India). The results of this study could be a step towards development of a new variety of fennel seeds. Cluster analysis provided three clusters of respective fennel seeds depending on their antioxidant potential and phytochemical parameters. This study provides the evidence for further statistical approaches to identify the active components responsible for pharmacological activity.

Conflict of interest

The authors declare no conflicts of interest.

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