International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(5): 2292-2297

Received: 09-07-2017
Accepted: 10-08-2017

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Genetic variability for essential oil, polyphenols and antioxidant activity of coriander (*Coriandrum sativum* L.) genotypes grown in humid south eastern plain zone V of Rajasthan

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Abstract
Essential oil content, Total phenolic, flavonoid content and antioxidant activity of crude methanol extract were determined in twenty eight coriander genotypes grown in Humid South Eastern Plain Zone V of Rajasthan during Rabi 2016-2017. Essential oil content ranged from 0.24-0.75%. Highest essential oil was found in the entry Cor-122 (0.75%) at par to National check RCr-728 (0.74%). Total phenolics ranged from 126-555 GAE g^{-1}. Maximum phenolic content (555 mg GAE g^{-1}) was observed in Cor-144. Total flavonoids ranged from 64-455 mg QE g^{-1} seed. Highest total Flavonoid content was observed in Cor-136 (455.83 mg QE g^{-1} seed). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging antioxidant activity expressed as IC_{50} value ranged from 3.10-7.57 mg ml^{-1}. Lowest IC_{50} value was reported in Cor-142 showing that it has potential antioxidant activity over Hisar Anand and RCr-728. Current study reveals that coriander genotypes grown in Humid South Eastern Plain Zone V of Rajasthan contains adequate amount of variability for secondary metabolites and antioxidant activity.

Keywords: Coriander, Phenolics, Flavonoid, Antioxidant, 2, 2-diphenyl-1-picrylhydrazyl

Introduction
Coriander (*Coriandrum sativum* L.) is a culinary and medicinal plant from the Apiaceae family and cultivated throughout the sub-continent for both seed as well as tender leaves [1]. Rajasthan, Gujarat, Andhra Pradesh, Uttar Pradesh, Madhya Pradesh and Himachal Pradesh are the major coriander growing states in India [2].

The Coriander essential oil (EO) and extracts possess promising antibacterial, antifungal and anti-oxidative activities as various chemical components in different parts of the plant, which thus play a great role in maintaining the shelf-life of foods by preventing their spoilage [3].

The *C. sativum* oil from fully ripe and dried seeds is a colourless or pale yellow liquid with a characteristic odour and mild, sweet, warm and aromatic flavour, and linalool is its major constituent [4]. The main components of the volatile oil from the fresh herb are aliphatic with their unpleasant odour [5]. The essential oils and extracts of aromatic plants and spices have been used in food preservation, pharmaceuticals, alternative medicine and natural therapies [6]. Since the EO contents in different species varies inherently, influenced greatly by culture conditions and environment, as well as by crop and post-crop processing, hence evaluations of the oils from many medicinal plants are being conducted [7-10].

Secondary metabolites or phytochemicals, naturally occurring in plants are biologically active and play important role in defense system of plants [11]. These phytochemicals have historically been used as pharmaceuticals, fragrances, flavor compounds, dyes, and agrochemicals [12]. *In vitro* studies reported that phytochemicals such as phenolic compounds have potential role against different diseases and used as anti-inflammatory, anti-mutagenic, antiviral and antibacterial, agents [13-14]. The phenolic and flavonoid content may contribute directly to the anti-oxidant activity [15].

Keeping the above facts in view, the present study was undertaken to analyze crude seed extracts of different genotypes of coriander for their secondary metabolites antioxidant potential as well as essential oil content for their suitability for pharmaceutical use.

Materials and Methods
Twenty eight coriander genotypes grown at Agricultural Research Station, Ummedganj, Kota in Humid South Eastern Plain Zone V of Rajasthan during *Rabi* 2016-2017 were used for

analysis. The study was conducted at Sanitary and Phytosanitary Laboratory, Agricultural Research Station, Mandor, (Agriculture University Jodhpur).Crude extracts prepared in methanol solvents were analyzed for total phenolic, total flavonoid contents as well as antioxidant activity in respect of DPPH radical scavenging IC_{50} value.

Chemicals and Reagent

The chemicals used in this study were procured from Loba Chemi (India) and Sigma-Aldrich (USA).

Estimation of Essential oil

28 genotypes of coriander seeds were collected from Agricultural Research Station Ummedganj Kota Rajasthan. The essential oil was extracted using hydro-distillation (HD) in Clevenger-type apparatus [16]. Fifty gram powdered sample was used to essential oil extraction. The essential oil content was calculated as a relative percentage (v/w). The results were calculated as means of triplicate.

Total phenolics content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu assay according to procedure describe by Dewanto *et al.* [17] with slightly modification and the results were expressed as mg gallic acid g^{-1} seed. An aliquot of 1 ml of the crude seed methanol extract was mixed with 1 ml of the Folin-Ciocalteu reagent and 4 ml of a 20 % sodium carbonate solution. Distilled water was added to a final volume of 25 ml. Following incubation for 30 min, the absorbance of the reaction mixture was measured at 765 nm using Lab India make spectrophotometer against a blank. Gallic acid was used as the standard. The amount of total phenolic was calculated by using the standard curve of gallic acid (Figure-1) drawn within a concentration range of 8.0 x 10^{-4} to 4.0 x 10^{-3} mg ml^{-1} having R^2 value 0.994 and was expressed as mg Gallic acid equivalents g^{-1} (mg GAE g^{-1}) seed.

Total flavonoid content (TFC)

The total flavonoid content in methanol extract was determined using aluminium trichloride ($AlCl_3$) method, protocol described by Chang *et al.* [18] with slight modification. Briefly, 2 ml of 2 % aluminium trichloride ($AlCl_3$) solution in methanol was mixed with 2 ml of a diluted stock solution (0.01 or 0.02 mg ml^{-1}). Absorption readings were taken at 415 nm (Lab India spectrophotometer) after 10 min against a methanol blank, Quercetin was used as the standard. The total flavonoid content was determined using a standard curve (Figure-2) of Quercetin drawn within a concentration range of 4.0 x 10^{-3} to 2.0 x 10^{-2} mg ml^{-1} having R^2 value 0.996 and was expressed as mg Quercetin equivalents g^{-1} seed (mg QE g^{-1} seed).

Antioxidant activity DPPH assay

There are several methods commonly used to determine the antioxidant activity of natural products, however 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical reagent was choosen in the present study as it is an easy, precise, and accurate method. 2, 2- diphenyl-1-picryl-hydrazyl DPPH is a free radical, and produces a violet solution in alcohol. It is reduced in the presence of an antioxidant molecule. Antioxidant activity of the methanol extract of coriander seed and standard were assessed on the basis of the radical scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl hydrate radical (DPPH). The diluted working solutions of the test samples were prepared in methanol. Gallic acid was used as the standard in solutions ranging from 5 x 10^{-4} to 4 x 10^{-3} mg ml^{-1}. 0.135 mM DPPH solution was prepared in methanol. Then 2 ml of this DPPH solution was mixed with 2 ml of sample solutions (ranging from 1 mg ml^{-1} to 10 mg ml^{-1}) and the standard solution was tested separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Lab India make spectrophotometer against methanol. 2 ml of methanol with 2 ml of DPPH solution was used as control [19-20]. The optical density (O.D.) was recorded and percentage of inhibition was calculated using the formula given below:

$$\% \text{ of inhibition of DPPH activity} = \frac{\text{O.D. of the control- O.D. of the sample x 100}}{\text{O.D. of the control}}$$

The IC_{50} values were calculated using linear regression of plots where the abscissa represented the concentration of the test solution and the ordinate was the percent of antioxidant activity.

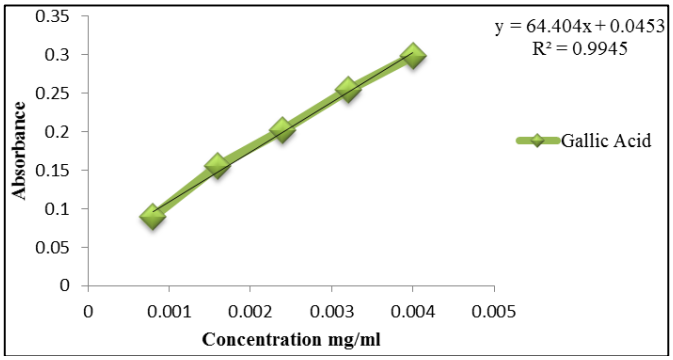

Fig 1: Gallic Acid Standard Linear Calibration Curve for Assessment of Total Phenolics

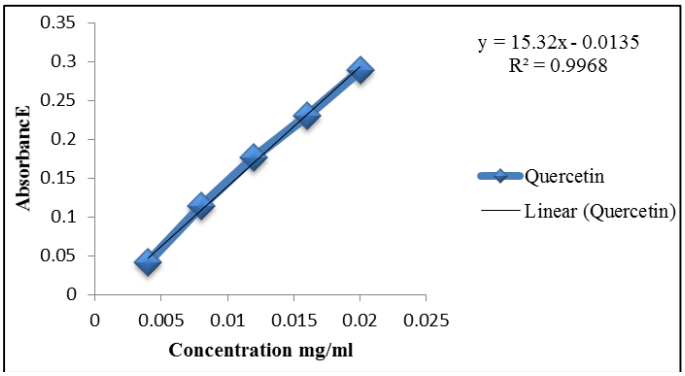

Fig 2: Quercetin Standard Linear Calibration Curve for Assessment of Total Flavonoids

Result and Discussion

Essential oil yield

Oil estimation studies indicated that the essential oil yield among 28 entries varies from 0.24 -0.75%. (Table-1) Cor-122 found superior over National checks Hisar Anand and RCr-728. Cor-123 (0.65%), Cor-124 (0.69%), Cor-128 (0.63%) and Cor-147 (0.65%) showed significant oil % as compared to Hisar Anand and RCr-728, implying their superiority in terms of quality. Cor-144 (0.24%) contained lowest essential oil among all genotypes while rest of the entries had insignificant essential oil content over Hisar Anand and RCr-728.

Earlier researchers have reported a large variation in essential oil yield of coriander. Ramezani *et al.* [21] reported 0.14-0.37% essential oil at different maturity stage of coriander seeds. Doshi *et al.* [22] reported 0.45 % essential oil yield in

Rajasthan variety Pratap Raj Dhaniya-1 while Ravi *et al.* [23] reported 0.82% essential oil yield in coriander seed. An environmental condition, processing technology and genetic constituents influences the yield of essential oil [1, 24]. Our study also confirms significant variation in coriander seed essential oil yield with respect to different genotypes.

Total Phenolics Content (TPC)

The colorimetric method using the Folin-Ciocalteau reagent is frequently used for total phenolic content estimation. A blue colour complex forms due to the reaction of Folin-Ciocalteau reagent and phenols that allow quantification.

Total phenolics content in coriander genotypes were ranged from 126-555 mg GAE g^{-1} (Table-1). Maximum phenolic content (555.70 mg GAE g^{-1}) was observed in Cor-144 followed by Cor-143 (538.90 mg GAE g^{-1}), RCr-728 (527.83 mg GAE g^{-1}), Cor-147 (490.34 mg GAE g^{-1}) and Hisar Anand (463.47 mg GAE g^{-1}). A large genetic variation was observed in total phenolics content among twenty eight genotypes. Lowest total phenolic was recorded in Cor-128 (126.06 mg GAE g^{-1}). Figure-3 showed comparative total phenolics among twenty eight genotypes.

Saxena *et al*. (2015) [1] analyzed total phenolics in different plant parts of coriander in different solvents and reported highest phenols in distilled water extract of root (50.141 mg GAE g^{-1}) while in methanol extract maximum phenols was in dried stem (47.328 mg GAE g^{-1}). Nagella *et al*. [25] analyzed four solvent extract of coriander seed and reported that ethyl acetate extract had the highest (23.09 mg/g) amount of phenolic compounds followed by butanol (21.95 mg/g), methanol (8.77 mg/g) and water extract (8.23 mg/g). Wangensteen *et al*. [26] also reported that ethyl acetate extract had the highest content of phenolic compounds in coriander from Norway. The present study recorded higher phenolics content in coriander genotypes over previous studies.

Total Flavonoid Content (TFC)

Total flavanoids content was estimated by aluminium trichloride method. Quercetin dihydrate was taken as standard flavonoid and results were calculated as means of triplicate and represented as mg Quercetin equivalent g^{-1} seed (mg QE $g^{-1)}$ with standard deviation.

Table-1 shows total flavonoids content in different genotypes of coriander expressed as mg QE g^{-1} seed. Comparative total flavonoids among twenty eight genotypes are shown in figure 4. Total flavonoids were ranged from 64-455 mg QE g^{-1} seed. Earlier, Nagella *et al*. [25] reported 0.26 to 1.08 mg QE g^{-1} in ethyl acetate, butanol, methanol and water extract of coriander seed.

Highest total Flavonoid content was observed in Cor-136 (455.83 mg QE g^{-1}) followed by Cor-124 (398.78 mg QE g^{-1}) and Cor-143 (360.90 mg QE g^{-1}) while National check Hisar Anand and RCr-728 had 261.81 and 286.52 mg QE g^{-1} respectively.Cor-124, Cor-125, Cor-136, Cor-137, Cor-141 and Cor-143 showed higher flavonoids as compared to National check Hisar Anand and RCr-728. Rest of the genotypes was found lower in flavonoids content. Lowest flavonoids content was found in Cor-128, Cor-147 and Cor-129.

Flavonoids have been shown to have a wide range of biological and pharmacological activities in *in vitro* studies. Examples included anti-allergic, anti-inflammatory antioxidant [27], anti-microbial antibacterial [28], antifungal, and antiviral, anti-cancer, and anti-diarrheal activities [29].

Antioxidant activity DPPH Assay

The determination of the antioxidant activity of coriander seed methanol extract was based on the DPPH radical scavenging activity through the IC_{50} parameter, which represents the concentration of the material necessary to inhibit 50% of free radicals. Thus, a lower IC_{50} value shows a superior ability to neutralize free radicals and potential antioxidant content.

The scavenging ability of methanol seed extract of coriander represented as IC_{50} of DPPH radical are shown in Table-1 while scavenging percentage in different concentration of extract was presented in table-2. Lowest IC_{50} value (3.10 mg/ml) was recorded in Cor-142 which is lower than national check Hisar Anand (6.28 mg/ml) and RCr-728 (4.91 mg/ml).

Saxena *et al.* [30] reported 10.24-12.03 mg/g antioxidant activity in methanol crude extract of coriander variety ACr-1different plant parts. Current study reveals that Humid South Eastern Plain Zone V growing genotypes contains lower IC_{50} value which indicates that they have potential antioxidant activity. Figure-5 showed comparative IC_{50} value among twenty eight genotypes. Higher phenolic and flavonoids are indicating the higher antioxidant activity which reflects in current study.

Table 1: Essential Oil, Phenolics, flavonoid and Antioxidant Activity of Coriander genotypes

S. No.	Genotype	Essential Oil %	Total Phenolics mg GAE g^{-1} Seed	Total Flavonoid mg QE g^{-1} Seed	Antioxidant IC_{50} Value mg/ml
1.	COR 122	0.75 ± 0.08	176.05 ± 2.44	144.29 ± 1.66	7.57
2.	COR 123	0.65 ± 0.12	192.33 ± 1.15	134.40 ± 1.55	4.94
3.	COR 124	0.69 ± 0.07	285.78 ± 1.82	398.78 ± 1.54	4.96
4.	COR 125	0.38 ± 0.11	228.35 ± 1.24	295.38 ± 1.44	4.14
5.	COR 126	0.42 ± 0.05	386.78 ± 4.49	148.04 ± 3.76	4.38
6.	COR 127	0.33 ± 0.10	205.32 ± 3.60	240.81 ± 3.68	6.08
7.	COR 128	0.63 ± 0.08	126.06 ± 1.49	64.52 ± 1.63	5.12
8.	COR 129	0.47 ± 0.09	189.98± 3.67	86.38 ± 1.04	4.93
9.	COR 130	0.39 ± 0.09	231.09 ± 4.66	146.63 ± 2.86	4.11
10.	COR 131	0.43 ± 0.07	349.90 ± 1.25	112.43 ± 2.54	5.09
11.	COR 132	0.37 ± 0.05	307.58 ± 5.52	188.57 ± 1.51	4.66
12.	COR 133	0.39 ± 0.03	226.15 ± 1.61	163.79 ± 1.61	3.71
13.	COR 134	0.29 ± 0.09	259.96 ± 4.16	197.31 ± 2.91	7.24
14.	COR 135	0.35 ± 0.07	225.01 ± 3.93	144.29 ± 4.26	5.23
15.	COR 136	0.36 ± 0.04	376.39 ± 4.50	455.83 ± 2.85	4.77
16.	COR 137	0.38 ± 0.10	395.06 ± 4.62	304.82 ± 4.53	4.65
17.	COR 138	0.39 ± 0.11	407.52 ± 2.21	247.69 ± 4.36	4.66
18.	COR 139	0.36 ± 0.05	330.41 ± 4.38	212.84 ± 3.29	3.89

19.	COR 140	0.43 ± 0.15	405.26 ± 3.50	176.26 ± 1.03	3.53
20.	COR 141	0.48 ± 0.09	458.99 ± 3.01	263.72 ± 3.59	3.15
21.	COR 142	0.27 ± 0.05	410.89 ± 4.47	220.80 ± 1.60	3.10
22.	COR 143	0.33 ± 0.16	538.90 ± 1.07	360.90 ± 1.76	4.74
23.	COR 144	0.24 ± 0.07	555.70 ± 3.34	225.99 ± 1.46	5.31
24.	COR 145	0.28 ± 0.09	408.65 ± 4.49	163.01 ± 2.34	4.87
25.	COR 146	0.40 ± 0.06	319.67 ± 0.28	77.58 ± 1.55	4.51
26.	COR 147	0.65 ± 0.11	490.34 ± 2.11	84.79 ± 5.83	6.29
27.	Hisar Anand (NC)	0.71 ± 0.08	463.47 ± 0.76	261.81 ± 1.56	6.28
28.	RCr 728 (NC)	0.74 ± 0.04	527.83 ± 2.98	286.52 ± 1.96	4.91

Mean ± SD

Table 2: DPPH radical scavenging % in crude extract of coriander genotypes

S. No.	Genotype	DPPH Radical Scavenging % at Different Concentration of Crude Seed Methanol Extract		
		1 mg/ml	5 mg/ml	10 mg/ml
1.	COR 122	6.49	32.66	66.22
2.	COR 123	12.75	58.61	88.37
3.	COR 124	15.88	57.94	84.56
4.	COR 125	27.52	57.49	89.49
5.	COR 126	24.16	57.72	88.14
6.	COR 127	14.09	44.97	75.62
7.	COR 128	14.32	53.98	86.80
8.	COR 129	15.66	55.77	88.37
9.	COR 130	27.29	60.18	86.58
10.	COR 131	10.74	57.34	88.14
11.	COR 132	21.92	55.77	86.80
12.	COR 133	27.74	66.44	88.14
13.	COR 134	14.73	42.42	62.69
14.	COR 135	14.20	49.05	89.39
15.	COR 136	16.86	53.22	94.51
16.	COR 137	19.51	52.84	94.89
17.	COR 138	10.98	60.98	97.25
18.	COR 139	28.45	58.14	95.83
19.	COR 140	30.68	61.74	98.11
20.	COR 141	33.71	64.96	98.23
21.	COR 142	37.12	60.80	96.21
22.	COR 143	23.33	51.33	88.17
23.	COR 144	16.17	52.00	82.50
24.	COR 145	14.83	57.17	89.33
25.	COR 146	16.50	64.17	89.00
26.	COR 147	18.66	48.66	67.33
27.	Hisar Anand (NC)	15.66	47.66	69.83
28.	RCr 728 (NC)	15.83	57.17	86.83

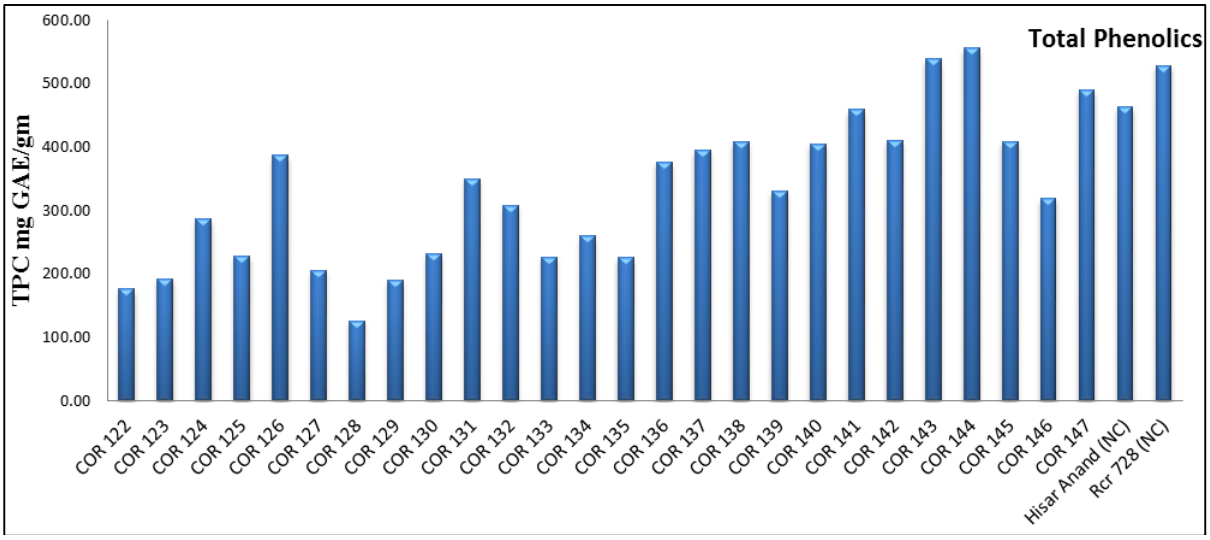

Fig 3: Total Phenolics content in coriander genotypes expressed as gallic acid equivalent g^{-1} seed

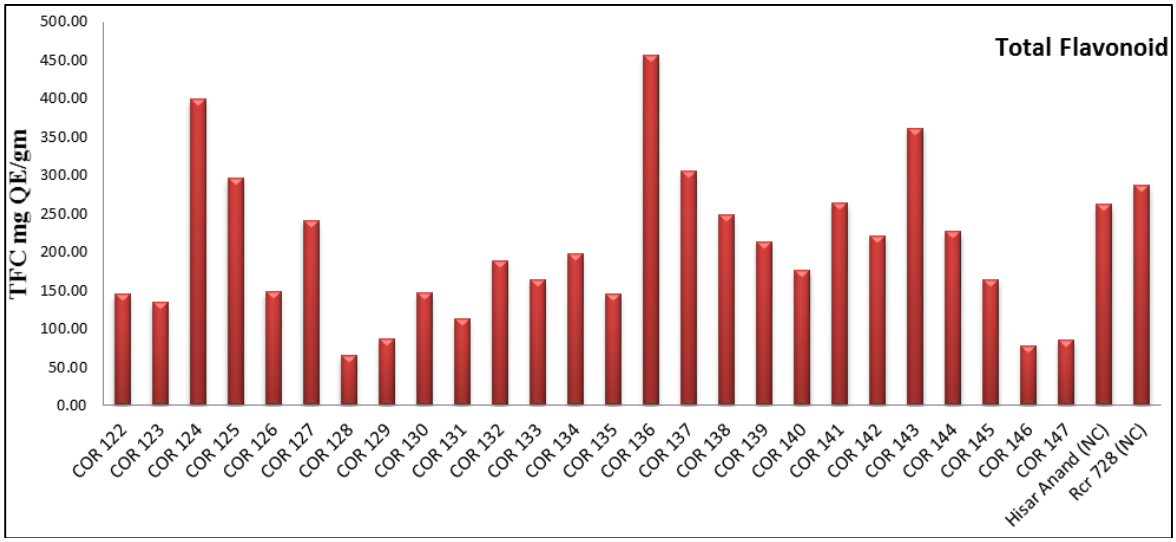

Fig 4: Total flavonoid content in coriander genotypes expressed as quercetin equivalent g^{-1} seed

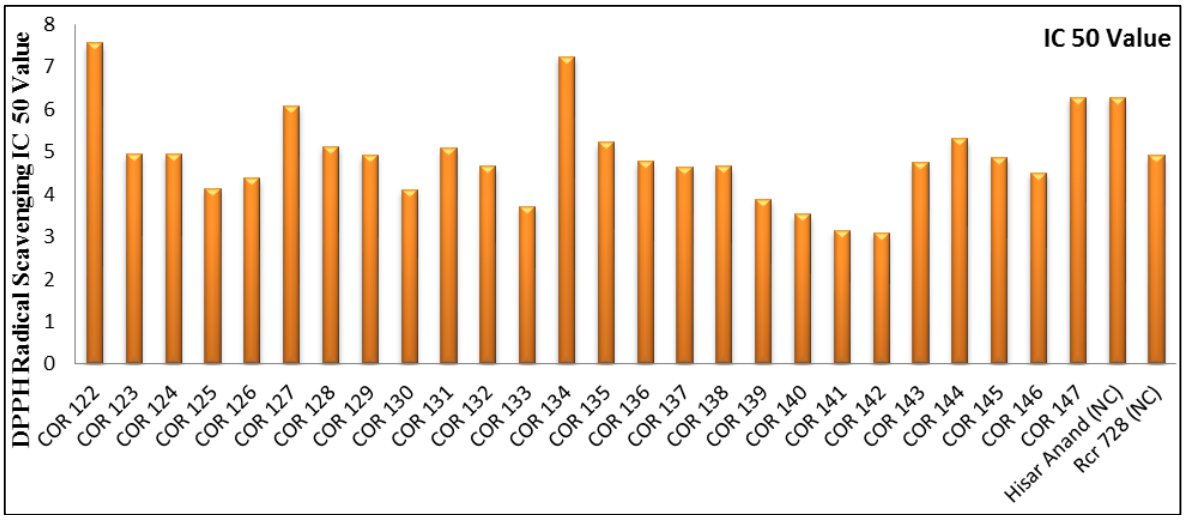

Fig 5: DPPH radical scavenging IC_{50} value (mg ml^{-1}) in coriander genotypes

Conclusion

The essential oil is responsible for the characteristic coriander odor. The phenolic and flavonoid content contribute directly to the anti oxidant activity. While measuring antioxidant activity and total phenolic content of some Asian vegetables, Kaur & Kapoor [31] categorized coriander in moderate or low phenolic containing vegetable group, but found very high anti-oxidant activity. In the present study, however we observed reasonably good amount of total phenolic content and high antioxidant activity in coriander seed methanol extract. Humid South Eastern Plain Zone V of Rajasthan of India is highly suitable for production of quality coriander. Cor-122 gives higher EO yield but poor in secondary metabolites content while entry Cor-124 contains significant amount of EO as well as secondary metabolites and antioxidant activity. National checks Hisar Anand and RCr-728 were perform better with respect to EO yield as well as secondary metabolite content. The information provided by this research will help breeders in future breeding programmes to develop improved varieties of coriander.

Acknowledgement

Authors are thankful to Dr. K. Nirmal Babu, Project Coordinator, All India Coordinated Research Project on Spices, Indian Institute of Spices Research, Calicut (Kerala) for financial grant for this study and Zonal Director Research, Agricultural Research Station, Mandor, (Agriculture University Jodhpur) for providing necessary research facilities.

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