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Genetic variation in phenolics and antioxidant content in seed and leaf extracts of coriander (*Coriandrum sativum* L.) genotypes

D. Agrawal[1], L. K. Sharma, M.K. Shirsat[2] and S. N. Saxena*

ICAR-National Research Centre on Seed Spices, Tabiji, Ajmer

[1]Ph.D. Scholar, Pacific University, Udaipur

[2]Pacific University, Udaipur

Abstract

Phenolic, flavonoid and antioxidant activity of seeds and leaves extract in different organic solvent and distilled water of five coriander genotypes were analyzed. Data revealed that substantially good amount of phenolics were present in seeds as well as leaves of coriander. All three solvents (Dimethylsulphoxide, hexane and methanol) and distilled water (DW) were effective in extracting phenolics from seeds and leaves of coriander genotypes. Genotypic variation was significant except hexane extract where non significant differences were observed in total phenolics both in seed and leaf extract. Leaf extract in DMSO showed more phenolics than seed extract in all the genotypes except RCr 436. Total flavonoid content in coriander genotypes differ significantly except methanol extract of seeds and leaves. Mean value indicated that hexane seed extract recovered highest TFC followed by methanol, DW and DMSO. TFC content was considerably higher in leaf extract of all the genotypes irrespective of the solvents. Hexane solvent extract showed highest TFC ranged from a minimum (59.34) in Azad Dhania-1 to maximum (65.50 mg QE gm^{-1}) in Gcr-1 followed by methanol (20.27), DMSO (19.79) and DW extract (8.10 mg g^{-1} leaves). All genotypes were significantly differing in there antioxidant content. Distilled water showed maximum amount of total antioxidant ranged from a minimum of 0.49 in genotype ACr-1 to a maximum of 0.57 mg BHTE gm^{-1} in genotype Azad Dhania-1 with DPPH Scavenging % with a mean value of 63.88% followed by DMSO (58.70%), Hexane (47.20%) and Methanol seed extract (17.48%). A reasonable relationship was also observed in phenolic content and antioxidant activity of seed as well as leaves of coriander.

Key words : Antioxidant, coriander, seed extract, total phenolic content, total flavonoid content

Introduction

Herbs and spices have been extensively used as food additives for natural antioxidants. Spices and aromatic herbs are considered to be essential in diets or medical therapies for delaying aging and biological tissue deterioration (Frankel, 1996). The search for naturally occurring antioxidants as alternatives of synthetic antioxidants is of great interest both in industry as well as in scientific research. It has been mentioned the antioxidant activity of plants might be due to their phenolic compounds (Cook and Samman, 1996). Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Frankel, 1996). Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski *et al*., 1987). The search of new antioxidants and phenolics from herbal source has taken very large attention in last decade. Antioxidant and antimicrobial properties are responsible for well being of human body hence, they are very much important for further characterization of plant material. Secondary metabolites from plants, mainly phenolics having antioxidants, antimicrobial, antitumour, antiviral, enzyme inhibiting and radical scavenging properties (Sengul, 2009). Isolation of antioxidant compounds from plants is possible through extraction with different solvents and it depends on the nature of extracting solvents (Bushra *et al*., 2009). The extracts from plants contain different classes of phenols, which have the different solubility in different solvents.

Coriander (Coriandrum sativum L.) is an annual herb in the family Apiaceae. It is also known as Chinese parsley or, particularly in the Americas, cilantro. It is native to southern Europe and North Africa to south western Asia. India is largest producer and exporter of coriander in the World. It is mainly cultivated in Rajasthan, Andhra Pradesh, Gujarat and Madhya Pradesh with scattered pockets in Tamil Nadu, Orissa, Karnataka, Haryana, Uttar Pradesh and Bihar. Coriander is an important spice crop having a prime position in flavouring food. The whole plant has a pleasant aroma. Coriander seeds are reported to have phenolics and good antioxidant properties (Saxena, 2015). Similarly, coriander leaves also showed good

*Corresponding author email: shail.nrcss@gmail.com

antioxidant potential (Saxena *et. al*., 2016). Present investigation was carried out to evaluate the genetic variation in total phenolics, flavonoid and antioxidant properties of five selected coriander genotypes.

Material and methods

Seeds of five released varieties of coriander ACr-1, Azad Dhania-1, GCr-1, Hisar Sughandh and Rcr-436 were obtained from seed store of ICAR-NRCSS, Ajmer. Obtained seeds were cleaned and used for preparing extracts of different organic solvents and distilled water. Plants were raised for their leaves and further extracts were prepared from leaves at flowering stage in methanol, DMSO, hexane and distilled water. Total phenolic, flavonoids and antioxidant content, DPPH scavenging percentage and EC_{50} value of seed and leaves extract in different solvents were analyzed.

The dried seeds (30 gm) of each variety were ground to fine powders separately, by milling. The resulting materials were extracted with hexane, methanol, distilled water and DMSO using Accelerated Solvent Extractor (Dionex India Pvt. Ltd.). Extraction in distilled water was done manually in a mortar and pestle after soaking the seeds for overnight. Extraction was repeated three times and supernatants were pooled for further analysis. Final concentration was adjusted to 5mg/ml of seed material. These diluted extracts were used for determination of the total phenol and flavonoid concentration, as well as antioxidant content, antioxidant activity and EC_{50}.

Total Phenol Content (TFC) was determined using Folin-Ciocalteu assay, as described by Amin (2006). An aliquot of 0.1ml extract (5 mg ml^{-1} in respective solvent) was taken in a test tube and made the volume 1ml by adding solvent. 3ml of 10% sodium carbonate was added. Previously 10-fold diluted Folin- Ciocalteu reagent was added to the mixture. The mixture was allowed to stand at room temperature for 90 minutes and then absorbance was measured at 710 *nm*. Gallic acid was used as the standard phenol. The amount of phenolic content was calculated by using the standard curve of Gallic acid prepared with respective solvent having R^2 value ranged from 0.96-0.99 and was expressed as mg Gallic Acid Equivalents GAE g^{-1} seed / leaves.

Total Flavonoid Content (TFC) was determined by using previously reported method by Chang *et al.,* (2002) with slight modification. One ml of suitably diluted sample was taken in a test tube and 100 µl aluminum chloride (1M) solution was added carefully from the side wall of the test tube followed by addition of 100 µl potassium acetate. The total volume was made 4 ml by adding 2.8 ml of solvent in the test tube. After 30 minute incubation of reaction mixture at room temperature stable Yellow color was developed. Absorbance was measured at 517 *nm*. Quercetin was used as the standard flavonoids. The amount of flavonoid was calculated by using the standard curve of Quercetin prepared with respective solvent having R2 value ranged from 0.96-0.99 and was expressed as mg Quercetin Equivalents QE g^{-1} seeds / leaves.

The antioxidant activity of each extract was evaluated on the basis of its activity in scavenging the stable DPPH (1, 1-Diphenyl-2-picrylhydrazin) radical, using a slight modification of the method described by Shimada (1992). Each extract was diluted in methanol/ Hexane / DMSO and distilled water to give at least 5 different concentrations. An aliquot (1, 1.5, 2, 2.5 ml) of the extract of each concentration was mixed with 1 ml of 1 M DPPH. The mixture was then homogenized and left to stand for 30 min in the dark. The absorbance was measured at 517 *nm* against a blank of methanol using a spectrophotometer. DPPH solution plus methanol was used as control and Butyl hydroxyl toluene (BHT) was used as a standard reference synthetic antioxidant with R^2 value ranged from 0.95- 0.99. Results were expressed as a mean standard deviation from three replicate measurements. The percent scavenging effect was calculated using following equation:

$$Scavenging\ effect\ (\%) = \frac{A_{517}\ of\ control - A_{517}\ of\ extract}{A_{517}\ of\ control} \times 100$$

The EC_{50} value for each sample defined as the concentration of the test sample leading to 50% reduction of the DPPH concentration was calculated from the non linear regression curve of the test extract.

Results and discussion

Estimation of Total Phenolic Content (TPC)

Total phenolic content extracted using different solvents from seeds and leaves of coriander genotypes and expressed as mg Gallic Acid Equivalent are given in Table 1. Data revealed that substantially good amount of phenolics were present in seeds as well as leaves of coriander. All three solvents (DMSO, hexane and methanol) and distilled water were effective in extracting phenolics from seeds and leaves of coriander genotypes. Hexane seed extract showed maximum phenolics ranged from a minimum of 6.33 in RCr-436 to maximum of 7.15 mg GAE g^{-1} in ACr-1. Phenolic content was at par in methanol, DMSO and distilled water extract with mean value of 4.89, 5.13 and 4.89 respectively. Genotypic variation was significant except hexane extract where non significant differences were observed in total phenolics both in seed and leaf extract. Leaf extract in DMSO showed more phenolics than seed extract in all the genotypes except RCr 436. This increase was, however, less in methanol leaf extract. Distilled water extract of coriander leaves of all genotypes showed less amount of phenolics ranged from a minimum of 2.85 in ACr 1 to a maximum of 4.48 in Hisar Sugandha with a mean value of 3.73 mg GAE g^{-1} leaves

Total Flavonoid Content in seeds and leaves of coriander Genotypes was extracted in distilled water, DMSO, Hexane and Methanol and expressed in mg QE gm^{-1} plant material. Total flavonoid content in coriander genotypes differ significantly except methanol extract of seeds and leaves. Mean value indicated that hexane seed extract recovered highest TFC followed by Methanol, DW and DMSO. In Hexane extract amount of TFC was ranged from a minimum of 1.76 in GCr-1 to a maximum 2.05 mg QE gm^{-1} in Azad Dhania-1 followed by methanol seed extract where minimum (1.38) TFC was found in Hisar Sugandh and maximum (2.08 mg QE gm^{-1}) was in GCr-1. Distilled water and DMSO seed extract recovered 0.45 and 0.22 mg QE gm^{-1} seed respectively, however, significant genetic variation was observed for TFC in five coriander genotypes (Table 2).

TFC content was considerably higher in leaf extract of all the genotypes irrespective of the solvents. Hexane solvent extract showed highest TFC ranged from a minimum (59.34) in Azad Dhania-1 to maximum (65.50 mg QE gm-1) in Gcr-1 followed by methanol (20.27), DMSO (19.79) and D/W extract (8.10 mg g^{-1} leaves).

Estimation of antioxidant Activity

Antioxidant Activity in Seed of Coriander Genotypes

Distilled water, DMSO, Hexane and Methanol crude seed and leaf extracts of all coriander genotypes were evaluated for its antioxidant activity in terms of total antioxidant content, DPPH free radical scavenging % and EC_{50} value. All genotypes were significantly differing in there antioxidant content. Distilled water showed

Table 1. Total Phenolic Content (TPC) in DW, DMSO, hexane and methanol crude seed and leaf extract of coriander genotypes

Genotype	Total Phenolic Content (mg GAE g^{-1} seeds/ leaves)							
	Distilled Water		DMSO		Hexane		Methanol	
	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf
ACr-1	5.00	2.85	4.95	9.35	7.15	6.08	4.81	5.41
Azad Dhania-1	4.89	3.76	5.40	8.52	6.76	6.06	4.18	5.42
GCr-1	4.83	3.57	4.45	9.89	6.44	5.87	5.14	4.91
Hisar Sugandh	4.69	4.48	5.0	10.08	6.58	5.87	5.03	4.95
RCr-436	5.09	4.03	5.88	5.63	6.33	5.98	5.31	6.16
Mean	4.89	3.73	5.13	8.69	6.65	5.97	4.89	5.37
CD (0.05%)	0.18	0.16	0.15	1.12	NS	NS	0.40	0.441
CV	2.01	2.26	1.54	6.87	6.77	5.22	4.35	4.357

Table 2. Total Flavonoid Content (TFC) in D/W, DMSO, hexane and methanol crude seed and leaf extract of coriander genotypes

Genotype	Total Flavonoid Content (mg QE g^{-1} seeds/ leaves)							
	Distilled Water		DMSO		Hexane		Methanol	
	Seed	Leaves	Seed	Leaves	Seed	Leaves	Seed	Leaves
ACr-1	0.47	6.97	0.10	18.37	1.77	60.87	1.63	21.38
Azad Dhania-1	0.32	7.84	0.15	22.44	2.05	59.34	1.46	20.32
GCr-1	0.41	7.99	0.22	19.59	1.76	65.50	2.08	18.29
Hisar Sugandh	0.32	7.57	0.02	19.82	2.02	60.36	1.38	20.96
RCr-436	0.74	10.14	0.60	18.75	1.89	61.99	1.63	20.40
Mean	0.45	8.10	0.22	19.79	1.89	61.61	1.63	20.27
CD	0.121	0.880	0.083	0.831	0.131	3.843	NS	NS
CV	14.21	5.763	20.00	2.22	3.65	3.31	16.48	6.05

Table 3. Total Anti- oxidant Content (TFC) in DW, DMSO, hexane and methanol crude seed and leaf extract of coriander genotypes

Genotype	Anti- oxidant content (mg BHT E g^{-1} seeds)							
	Distilled Water		DMSO		Hexane		Methanol	
	Seed	Leaves	Seed	Leaves	Seed	Leaves	Seed	Leaves
ACr-1	0.49	1.83	0.50	2.91	0.38	2.54	0.10	2.82
Azad Dhania-1	0.57	2.11	0.49	2.88	0.38	2.59	0.15	1.79
GCr-1	0.53	2	0.49	2.89	0.34	2.49	0.34	2.84
Hisar Sugandh	0.54	1.91	0.48	2.90	0.41	2.60	0.15	2.80
RCr-436	0.56	1.54	0.50	2.85	0.44	2.55	0.11	2.84
Mean	0.53	1.87	0.49	2.88	0.39	2.55	0.17	2.61
CD	0.040	0.101	NS	NS	0.053	NS	NS	NS
CV	4.00	2.85	3.02	1.05	7.15	3.24	104.38	0.79

maximum amount of total antioxidant ranged from a minimum of 0.49 in genotype ACr-1 to a maximum of 0.57 mg BHTE gm^{-1} in genotype Azad Dhania-1. There is non significant genetic variation for antioxidant content in leaf extract of either DMSO or methanol. However, antioxidant content was more (2.88) in DMSO leaf extract followed by methanol (2.61), Hexane (2.55) and Distilled water (1.87 mg BHT E Gm^{-1} leaves).

DPPH Scavenging % was maximum in distilled water seed extract with a mean value of 63.88% followed by DMSO (58.70%), Hexane (47.20%) and Methanol seed extract (17.48%). In distilled water seed extract minimum (58.19) DPPH scavenging % was recorded in genotype ACr-1 while maximum (67.36) was in genotype Azad Dhania-1. Methanol seed extract showed minimum DPPH Scavenging % ranged from a minimum (14.4) in genotype ACr-1 to maximum (20.37) in genotype Hisar Sugandha.

Leaf extract of all coriander genotypes whether extracted in DMSO, hexane or methanol showed more DPPH scavenging % as compared to distilled water extract. DPPH scavenging % was maximum in DMSO seed extract with a mean value of (90.76%) followed by Methanol (82.59%) and Hexane (80.70%) seed extract Distilled water seed extract showed minimum DPPH Scavenging % ranged from a minimum (49.81) in genotype RCr-436 to maximum (67.37) in genotype Azad Dhania-1 Different organic solvents have different polarity and therefore have different nature to extract the compounds. Souri *et.al.,* (2007) used methanol for extraction of total phenol content in fenugreek and other plant species while Kaur and Kapoor (2002) used ethanol extract for measurement of antioxidant activity and total phenol content of some Asian vegetables. In present study,

Table 4. DPPH Scavenging % in DW, DMSO, hexane and methanol crude seed extract of coriander genotypes

Genotype	DPPH Scavenging (%)							
	Distilled Water		DMSO		Hexane		Methanol	
	Seed	Leaves	Seed	Leaves	Seed	Leaves	Seed	Leaves
ACr-1	58.19	58.65	59.8	91.54	45.93	80.24	14.4	88.62
Azad Dhania-1	67.36	67.37	58.61	90.45	46.21	81.85	20.02	57.65
GCr-1	63.72	63.88	58.05	90.74	41.73	78.82	17.78	89.41
Hisar Sugandha	63.79	61.14	57.07	91.32	49.64	82.02	20.37	88.00
RCr-436	66.38	49.81	60.01	89.78	52.52	80.57	14.84	89.29
Mean	63.88	60.17	58.70	90.76	47.20	80.70	17.48	82.59
SD	3.562	6.636	1.226	0.703	4.0856	1.306	2.799	13.95

however, maximum phenolic and flavonoid contents were observed in hexane seed extract while DMSO leaf extract showed maximum phenolics and methanol leaf extract showed maximum flavonoid in all studied genotypes.

Due to presence of different antioxidant components in plant tissues it is relatively difficult to measure each antioxidant component individually. Therefore several methods have been developed in recent years to calculate the total antioxidant activity of biological samples (Al-Saikhon *et al*. 1995). Different solvents have been tried by various workers for extraction of antioxidants from the samples (Kahkonen *et al*., 1999). In present study, seed and leaves extracts were prepared in four different solvent *viz.* methanol, DMSO, hexane and distilled water and screened for their possible antioxidant and radical scavenging activity by DPPH method. Distilled water seed extract showed more antioxidant than other organic solvents but in case of leaf extract more antioxidant activity was found in methanol and DMSO extracts.

The phenolic and flavonoid content may contribute directly to the anti oxidant activity (Awika *et al*., 2003, Saxena *et. al*., 2013). In present study also TPC and TFC content in different seed extract showed proportional relationship with DPPH scavenging percentage, a measure of antioxidant activity except methanol seed extract (Fig 1&2).

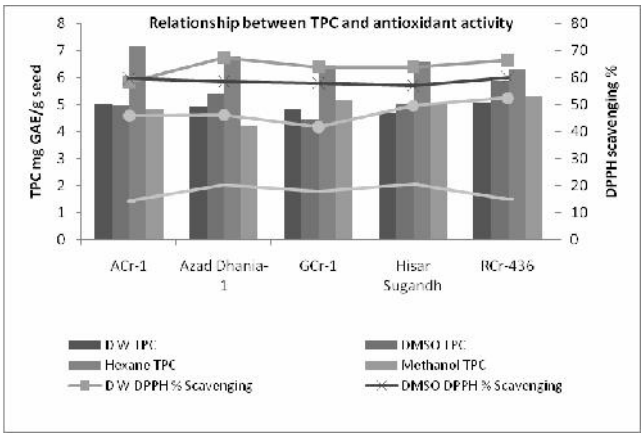

Fig. 1: Relationship between phenolic contents and anti oxidant activity in coriander genotypes

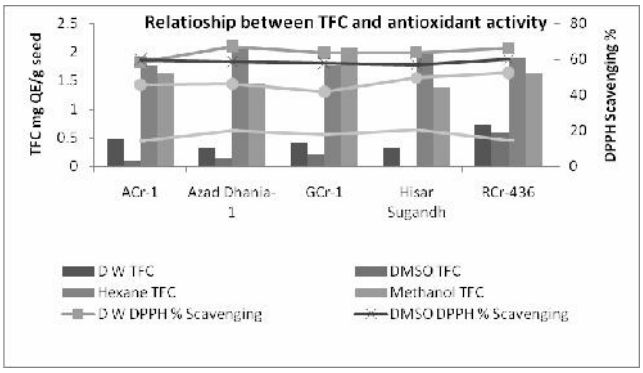

Fig. 2: Relationship between flavonoid contents and antioxidant activity in coriander genotypes

There are many reports in which showed low phenolic content but high antioxidant activity or vice versa. This can be explained on the basis of high anti-oxidant activity of some individual phenolic units, which may act as efficient antioxidants rather than contributing to high total phenolics. The scavenging action of various phenolic compounds is closely connected with their spatial conformation. Such results have been reported by Chu *et al*., (2000) in vegetables like white cabbage and crown daisy, which despite having low phenolic contents had moderate antioxidant activity. They attribute this to the presence of some other phytochemicals such as phenolic acid, ascorbic acid, tocopherol and pigments, which also contribute to total antioxidant activity. From the present work, it could be concluded that solvent used for extraction is very important for effective extraction of the plant constituents. Genotypic variability for antioxidant activity in coriander could be exploited as a potent source of natural antioxidant along with other medicinal properties. Further studies are needed for the isolation and identification of the active component in the extract.

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