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Analysis of total phenolics and antioxidant activity in seed and leaf extracts of cumin genotypes

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Abstract

Total phenolic, flavonoid, antioxidant content and 1, 1-Diphenyl-2-picrylhydrazin scavenging activity of seed and leaves extract in different organic solvent and distilled water of five cumin genotypes were analyzed. Maximum phenolic content was observed in methanol seed extract of all cumin genotypes with an average value of 85.64 mg GAE g^{-1} seeds while minimum TPC observed in genotype RZ 19 (70.46 mg GAE g^{-1} seeds). TFC content was ranging from a minimum of 38.05 in RZ 19 to a maximum of 55.07 mg QE g^{-1} seeds in RZ 341. Antioxidant content was found maximum (36.93 mg BHTE g^{-1} seeds) in DMSO seed extract of RZ 209, while minimum (10.58 mg BHTE g^{-1} seed) was observed in methanol extract of RZ 19. In leaves, total phenolic and flavonoid content was observed maximum (60.05 mg GAE g^{-1} and 462.22 mg QE g^{-1} leaves) in methanol extract of RZ 209 and GC 4 respectively. Methanol solvent was found more effective in extracting anti oxidant content from leaves of all studied genotypes followed by DMSO, hexane and distilled water extract. DPPH Scavanging (%) of seed extracts was maximum (86.9 %) in RZ 209 DMSO, while minimum (25.51 %) in methanol extract of RZ 19. Similarly, EC_{50} value was observed maximum (42.45 %) in GC 1 when extracted in DMSO, while minimum (19.11 %) in methanol extract of RZ 19. DPPH Scavanging (%) of leaves extract was maximum (94.85 %) in methanol extract of RZ and minimum (71.44 %) in distilled water extract of GC 1.

Key words: Antioxidant, cumin, 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 solubilities in different solvents.

Cumin (*Cuminum cyminum* L.) is a small annual

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and herbaceous plant belonging to the *Apiaceae* family. It is one of the popular spices regularly used as a flavouring agent. Cumin seeds are used as a spice for their distinctive aroma, popular in Indian, Pakistani, North African, Middle Eastern, Sri Lankan, Cuban, Northern Mexican cuisines, and the Western Chinese cuisines of Sichuan and Xinjiang (Daniel and Maria, 2002). Cumin seeds have been used for treatment of toothache, dyspepsia, diarrhoea, epilepsy, and jaundice (Nostro and others, 2005). Cumin (*Cuminum cyminum* L.**)** is one of the most important seed spices commonly grown in arid and semi arid region of India. In India it is mainly cultivated as r*abi c*rop in Rajasthan, Gujarat and in some parts of Madhya Pradesh and Uttar Pradesh. The purpose of this study was to evaluate different seed and leaf extracts of cumin by measuring total phenolic content and total flavonoid content and their possible relation with antioxidant properties.

Materials and methods

Seeds of five released varieties of cumin RZ 209, RZ 19, RZ 341, GC 1 and GC 4 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^{-1} 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 2.8ml 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 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.

Result and discussion

Total phenolic content expressed as mg Gallic Acid Equivalent and flavonoid content expressed as mg QE g^{-1} seed material in different seed extracts from five genotype of cumin are given in Table 1. Maximum phenolic content was observed in methanol seed extract of all cumin genotypes with an average value of 85.64 mg GAE g^{-1} seed followed by DMSO (70.55 mg GAE). Hexane and distilled water extract showed at par phenolic content (63.59 and 63.39 mg GAE g^{-1} seed respectively. total phenolic content in methanol extract of genotype RZ 209 was observed 95.10 mg followed by GC 4 (92.43), RZ 341 (85.87), GC 1 (84.36) while minimum TPC observed in genotype RZ 19 (70.46 mg GAE g^{-1} seed material). There was observed a significant genotypic variation in total phenolic content that depends on solvent used.

Similar trend was observed in total flavonoid content where methanol extract was able to extract maximum TFC, content of which was ranging from a minimum of 38.05 in RZ 19 to a maximum of 55.07 mg QE g^{-1} seed in RZ 341. After methanol, hexane and DMSO showed at par ability in extracting TFC. Hexane was found more effective in genotype RZ 19 and RZ 341 while DMSO extracted more TFC in RZ 209, GC 1and GC 4 (Table 1). Distilled water was least effective able to extract TFC minimum (11.42 mg QE) in RZ 341 and maximum14.73 mg QE g^{-1} seed in RZ 19.

Antioxidant content was found maximum in RZ 209 (36.93 mg BHTE g^{-1} seed material) when extracted in DMSO, while minimum (10.58 mg BHTE g^{-1} seed) AO content was observed in methanol extract of RZ 19. DMSO solvent was found more effective in extracting anti oxidant content from all studied genotypes. Contrary to TPC and TFC, antioxidant content was found at par with DMSO in distilled water extract and methanol was least effective in extraction of antioxidant content.

Total phenolic, flavonoid and antioxidant content was analyzed leaves of cumin plant at flowering stage when plant attained maximum leaves and enter in to reproductive stage. Crude leaf extracts were prepared in distilled water, methanol, DMSO and hexane and TPC, TFC, AO content were evaluated. Total phenolic content was observed maximum (60.057 mg GAE g^{-1} leaves) in RZ 209 when extracted in methanol, while minimum (7.82 mg GAE g^{-1} leaves) in distilled water extracted of RZ 341. As per mean TPC content maximum TPC (57.72 mg GAE g^{-1} leaves) was recorded in methanol solvent followed by hexane (51.22 mg GAE g^{-1} leaves), DMSO (40.63 mg GAE g^{-1} leaves) while mean TPC was found minimum (16.88 mg GAE g^{-1} leaves) in distilled water extract of all genotypes (Table 2).

Total flavonoid content was observed maximum (462.22 mg QE g^{-1} leaves) in GC 4 when extracted in methanol, while minimum (30.24 mg QE g^{-1} leaves) in distilled water extracted of RZ 341. As per mean TFC content maximum TFC (430.86 mg QE g^{-1} leaves) was recorded in methanol solvent followed by hexane (346.85 mg QE g^{-1} leaves), DMSO (313.37 mg QE g^{-1} leaves) while mean TFC was found minimum (54.92 mg QE g^{-1} leaves) in distilled water extract of all genotypes (Table 2).

Table 1. Total phenolic, flavonoid and antioxidant content in crude seed extract of cumin genotypes

Genotypes	Total Phenolic Content (mg GAE g^{-1} seeds)				Total Flavonoid Content (mg QEg^{-1} seeds)				Antioxidant Content (mg BHTE g^{-1} seeds)			
	Hexane	Methanol	Distilled water	DMSO	Hexane	Methanol	Distilled water	DMSO	Hexane	Methanol	Distilled water	DMSO
RZ 209	74.79	95.10	90.79	80.57	30.66	53.27	14.61	34.66	22.93	19.98	36.10	36.93
RZ 19	47.54	70.46	47.61	58.79	22.7	38.05	14.73	19.93	22.90	10.58	30.84	31.54
RZ 341	59.37	85.87	51.78	69.01	22.85	55.07	11.42	20.96	23.85	12.56	27.21	32.31
GC 1	79.95	84.36	47.71	72.50	25.82	46.28	14.51	32.29	21.54	11.82	33.45	35.79
GC 4	56.29	92.43	79.06	71.88	24.27	41.15	27.33	31.39	21.05	14.50	35.42	34.77
Mean	63.59	85.64	63.39	70.55	25.26	46.77	16.52	27.85	22.45	13.89	32.60	34.26
SEm (±)	3.49	2.13	4.42	3.57	0.57	2.57	1.71	0.51	0.87	1.13	0.75	0.48
CD	11.40	6.97	14.41	11.66	1.87	8.41	5.59	1.68	2.84	3.68	2.47	1.58
CV	9.52	4.32	12.07	8.78	3.93	9.55	18.72	3.21	6.71	14.08	4.02	2.46

Table 2. Total phenolic, flavonoid and antioxidant content in crude leaf extract of cumin genotypes

Genotypes	Total Phenolic Content (mg GAE g^{-1} leaves)				Total Flavonoid Content (mg QE g^{-1} leaves)				Antioxidant Content (mg BHTE g^{-1} leaves)			
	Hexane	Methanol	Distilled water	DMSO	Hexane	Methanol	Distilled water	DMSO	Hexane	Methanol	Distilled water	DMSO
RZ 209	55.39	60.05	22.66	38.41	369.46	399.35	69.85	322.50	122.44	151.06	103.21	133.40
RZ 19	44.37	56.68	12.77	43.58	335.16	429.54	42.76	336.19	120.05	149.55	120.30	130.59
RZ 341	49.82	58.93	7.82	41.22	348.26	425.71	30.24	274.21	123.42	146.95	128.13	137.03
GC 1	57.92	54.26	21.71	40.21	344.58	37.49	58.22	352.53	129.43	147.23	112.45	127.90
GC 4	48.64	58.70	19.46	39.76	336.78	462.22	73.53	281.42	126.83	150.58	115.53	142.78
Mean	51.22	57.72	16.88	40.63	346.85	430.86	54.92	313.37	124.43	149.08	115.92	134.30
SEm (±)	2.52	0.99	0.71	1.31	7.43	3.83	2.25	2.86	2.59	1.04	1.10	1.32
CD	8.24	3.25	2.32	4.28	24.26	12.51	7.33	9.35	8.47	3.39	3.59	4.30
CV	8.54	2.99	7.31	5.60	3.71	1.54	7.09	1.58	3.61	1.21	1.64	1.70

Antioxidant content was found maximum (151.06 mg BHTE g^{-1} leaves) in methanol leaves extract of RZ 209 while minimum (112.45 mg BHTE g^{-1} leaves) in hexane leaves extract of GC 1. Methanol solvent was found more effective in extracting anti oxidant content from leaves of all studied genotypes followed by DMSO, hexane and distilled water extract.

Table 3 and 4 showed DPPH scavenging percentage and EC_{50} value of crude seed and leaves extract in different solvents. In seed extracts, DPPH Scavanging (%) was maximum (86.9 %) in RZ 209 when extracted in DMSO, while observed minimum (25.51 %) in RZ 19 when extracted in methanol. Similarly, EC_{50} value was observed maximum (42.45) in GC 1 when extracted in DMSO, while observed minimum (19.11) in RZ 19 when extracted in methanol. When leaves were taken for study, DPPH Scavanging (%) was observed maximum (94.85 %) in RZ 209 when extracted in methanol, while it was minimum (71.44%) in GC 1 when extracted in distilled water. Similarly, EC_{50} value was observed more or less similar in all genotypes as well all solvent systems.

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, however, maximum phenolic contents were observed in methanol seed and leaves extract. There seems significant genotypic variation in TPC, TFC and antioxidant content as well as solvent used. Distilled water was found effective in extracting antioxidant content from seeds while methanol was good in case of leaves.

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

Table 3. DPPH scavenging % and EC_{50} of crude seed extract of cumin genotypes

Genotypes	DPPH Scavenging (%)				EC_{50} (mg BHTE)			
	Hexane	Methanol	Distilled water	DMSO	Hexane	Methanol	Distilled water	DMSO
RZ-209	55.04	48.31	85.01	86.9	20.83	20.22	21.23	21.24
RZ-19	54.97	25.51	73.03	74.64	20.83	19.11	21.11	21.13
RZ-341	57.14	31.44	64.77	76.40	20.87	19.98	21.00	21.14
GC-1	51.89	29.76	78.99	42.15	20.76	19.87	21.17	42.45
GC-4	50.77	35.85	83.47	82.00	20.73	20.26	21.21	21.20
SD (±)	2.58	8.73	8.29	17.59	0.05	0.46	0.09	9.51

Table 4. DPPH scavenging % and EC50 of crude leaf extract of cumin genotypes

Genotypes	DPPH Scavanging (%)				EC_{50} (mg BHTE)			
	Hexane	Methanol	Distilled water	DMSO	Hexane	Methanol	Distilled water	DMSO
RZ-209	77.5	94.85	65.87	84.14	78.99	79.63	78.33	79.27
RZ-19	76.04	93.93	76.38	82.44	78.93	79.6	78.94	79.2
RZ-341	78.04	92.36	80.94	86.34	79.02	79.55	79.14	79.35
GC-1	81.73	92.52	71.44	80.86	79.17	79.56	78.7	79.14
GC-4	80.15	94.56	73.3	89.82	79.11	79.62	78.8	79.47
SD (±)	2.24	1.149	5.61	3.50	0.09	0.03	0.30	0.12

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. Butyl Hydroxyl Toluene (BHT) was used as standard antioxidant.

The phenolic and flavonoid content may contribute directly to the anti oxidant activity (Awika *et al.,* 2003). In present study TPC and TFC content in different seed extract showed inverse relationship with DPPH scavenging percentage, a measure of antioxidant activity. Contrary to this leaves extracts showed positive relationship with antioxidant activity in all studied cumin genotypes.

Similar to the results of present study there are many reports in which low phenolic content material showing 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.

Similar 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. Since methanol is highly polar therefore able to extract more phenolic compounds. Genotypic variability for antioxidant activity in cumin 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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