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Estimation of antioxidant activity, phenolic and flavonoid content of cryo and conventionally ground seeds of coriander (*Coriandrum sativum* L.) and fenugreek (*Trigonella foenum-graecum* L.)

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ABSTRACT

Effect of cryogenic grinding on oleoresin content, total phenolics, flavonoid content and anti-oxidant properties of seed extract of two genotypes of coriander and fenugreek have been analyzed. Oleoresin content was significantly high in cryogenically ground samples. Total phenolic and flavonoids content was also high in both the genotypes of coriander and fenugreek. Methanol crude seed extract of all genotypes were evaluated for its antioxidant activity in terms of total antioxidant content and DPPH free radical scavenging %. DPPH scavenging % was invariably more in cryo ground seeds in all the genotypes. Higher concentration of antioxidant content and DPPH scavenging % suggested high antioxidant activity in cryo grinded samples. It could be concluded that cryogenic grinding technology is able to retain flavour and medicinal properties of coriander and fenugreek irrespective of the genotype.

Keywords: Antioxidant activity, *Coriandrum sativum*, *Trigonella foenum-graecum* L., Phenolic content, Flavonoid content.

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INTRODUCTION

Coriander (*Coriandrum sativum* L.) is an annual herb in the family Apiaceae while Fenugreek (*Trigonella foenum-graecum* L.) is an annual forage legume crop. In India coriander and fenugreek are major seed spice crops grown in throughout the sub continent either for green leaf purpose or for grains. Rajasthan, Gujarat, Andhra Pradesh, Uttar Pradesh, Madhya Pradesh, Himachal Pradesh are the major states growing coriander. India is the largest producer of fenugreek in the World. Rajasthan has maximum area and production of about more than 80% of India's total production.

Both seed spices are widely used for their medicinal properties specifically as anti oxidants. Fenugreek seeds have antioxidant activity and have been shown to produce beneficial effects such as neutralization of free radicals and enhancement of antioxidant apparatus.

It has been mentioned the antioxidant activity of

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plants might be due to their phenolic compounds (Cook and Samman, 6). 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, 7). Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski et al. 9). In the longer term, plant species (or their active constituents) identified as having high levels of antioxidant activity in vitro may be of value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals induced tissue damage.

The purpose of this study was to analyze total phenolic and flavonoid content, antioxidant properties and their possible Correlation.

MATERIALS AND METHODS

Seeds of two varieties of coriander (Sudha and RCr 436) and fenugreek (RMt 1 and RMt 305) were obtained from seed store of NRCSS. Cleaned seeds were used for grinding by conventional method and cryogenically. Ground seeds powder were used for estimation of oleoresin, essential ois and preparing methanol extract for estimation of anti oxidant properties. Cryo grinding of seeds was done using Cryo grinder at Central Institute for Post Harvest Engineering and Technology, Ludhiana. The Cryo ground powder was quickly packed using sealing machine and only opened at the time of analysis. For obtaining seed powder through conventional grinding dried seeds (30 gm) was ground separately by domestic mixer grinder.

Oleoresin content of coriander and fenugreek were extracted using Accelerated Solvent Extraction System (Dionex India Ltd.) with hexane as a solvent. The crude extracts in methanol were prepared manually in a mortar pestle after soaking the seeds of both the spices for overnight, extraction was repeated three times and supernatants were pooled for analysis. After collecting the supernatant of both the spices extracts were diluted to make a stock solution of known concentration. These diluted extracts were used for determination of the total phenolic, Flavonoid as well as antioxidant activities.

Total phenol concentration was determined using a Folin-Ciocalteu assay, as described by Amin et. al. (2) with slight modification. An aliquot of 0.1ml extract (5 mg/ml 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 R2 value ranged from 0.96-0.99 and was expressed as ppm Gallic acid equivalents (ppm GAE / 1000 ppm) crude seed extract.

Total flavonoids concentration was determined by using previously reported method by Chang et al. (4) with slight modification. One ml of suitably diluted sample was taken in a test tube and 100µl aluminum chloride (1M) solution was added carefully from the side wall of the test tube followed by addition of 100µl 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 ppm Quercetin equivalents (ppm QE / 1000 ppm) crude seed extract.

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 et. al. (14). Each extract was diluted in methanol 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 R2 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 as follows:

$$\text{Seavenging effect}\,(\%) = \frac{A_{517}\ \text{of control} - A_{517}\ \text{of Extract}}{A_{517}\ \text{of control}} \times 100$$

RESULTS AND DISCUSSION

Table 1 showed the effect of cryo grinding on recovery of oleoresin in coriander and fenugreek genotypes. Cryo ground samples showed more oleoresin percentage as

compare to non cryo ground samples of both coriander and fenugreek.

In coriander the oleoresin content from conventional ground seed was less (9.50%) in genotype Sudha as compare to RCR-436 (12.80 %) but cryo ground seeds powder exhibit significant increase in oleoresin percent being recovered 18.88% in RCR-436 and 18.53% in genotype Sudha. However in fenugreek cryo ground samples yielded more oleoresin than normal samples but the increase was not as much as in coriander. The oleoresin percent in conventionally ground seed powder was 4.6 % in RMT-1 and 4.8 % in RMT-305. While in cryo ground seeds oleoresin recovery in RMT-1 was 5.63 % and in RMT-305 it was 5.95%. The study provides the evidence of better quality product can be obtained using cryogenic grinding of spices.

In conventionally ground seeds of coriander TPC was significantly less (19.46 ppm GAE 1000^{-1} ppm crude extract) in Sudha genotype as compare to RCr 436 (62.39 ppm GAE1000^{-1} ppm crude extract). In cryogenically grounded sample TPC was significantly higher in both the genotypes being observed 63.61 and 87.08 ppm GAE 1000^{-1}ppm crude extract in genotype Sudha and RCr 436 respectively (Table 2).

Total Flavonoid Content was also more in cryo ground samples but the magnitude was less as compare to TPC. TFC in conventionally grounded seeds was 13.94 ppm QE1000^{-1} ppm extract in genotype Sudha to a maximum of 15.38 ppm QE1000^{-1}ppm extract in RCR-436 genotype (Table 2).

Similarly in fenugreek TPC was significantly more in cryogenically ground samples of both the genotype. It was 75.722 ppm GAE1000^{-1} ppm crude extract in RMT 1 and 83.544 ppm GAE 1000^{-1} ppm in genotype RMT 305. While TFC in conventionally ground seeds was 12.560 ppm QE 1000^{-1} ppm in genotype RMT 305 and 13.630 ppm QE 1000^{-1} ppm in RMT 1 genotype. TFC in cryo grounded samples was significantly more in both genotypes (Table 2).

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. 1, Gazzani et al. 8, Kahkonen et al. 10, Chu et al. 5, Tang et al. 16). The use of DPPH radical provides an easy, rapid and convenient method to evaluate the antioxidants and radical scavengers (Soler-Rivas et al. 15, Kansci et al. 11, Roginsky and Lissi, 13). Different solvents have been tried by various workers for extraction of antioxidants from the samples (Kahkonen et al. 10). In present study, seed extracts prepared in methanol and evaluated for its possible antioxidant and radical scavenging activity by DPPH method.

Table 3 presented the data on antioxidant content and DPPH Scavenging % in methanol crude extract from conventional and cryogenically grounded seeds of coriander genotypes. All genotypes significantly differing in there antioxidant content whether it is conventionally or cryogenically ground. In coriander Total antioxidant content in conventionally grounded seeds was 4.92 ppm BHT E 1000^{-1}ppm) and maximum 6.47 ppm BHT E1000^{-1} ppm).The amount of total antioxidant in cryo grounded seeds was significantly high in both the genotype of coriander (8.72 ppm BHT E 1000^{-1}ppm in RCr 436 and 10.21ppm BHT E 1000^{-1}ppm genotype Sudha.

Similarly in fenugreek, the amount of total antioxidant content in cryo ground seeds was significantly high in both genotype which was ranging from a minimum of (9.326 mg gm^{-1} BHT E 1000^{-1} ppm) in genotype RMT 1 to a maximum of (11.088 mg/gm BHT E 1000^{-1} ppm) in genotype RMT 305. Anti oxidant activity as measured by DPPH free radical scavenging % was significantly high in cryo ground seeds of both the genotypes of both coriander and fenugreek (Table 3).

The phenolic and flavonoid content may contribute directly to the anti oxidant activity (Awika et al. 3). While measuring antioxidant activity and total phenolic content of some Asian vegetables Kaur and Kapoor (12) categorized fenugreek in high phenolic contents vegetables group using ethanol as solvent and observed very high antioxidant activity. Contrary to this in present study we observed less TPC in fenugreek but high antioxidant. This may be the use of different organic solvent.

Similar to the results of present study there are many reports in which low phenolic content material showing high antioxidant activity. 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. (5) 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 present study it could be concluded that cryogenic grinding technology is superior to non cryogenic grinding of coriander and fenugreek for retention of flavour and medicinal properties of irrespective of the genotype. Almost all the genotypes from diverse origin showed significant increase in oleoresin content, total phenolics, flavonoids and anti-oxidant properties. The study provides the basis for the fact that coriander and fenugreek are being used as a potent natural source of anti-oxidant. Further, studies are needed for the isolation and identification of the active compound in the crude seed extract responsible for anti-oxidant activity.

Table 1., Effect of cryo grinding on recovery of oleoresin in coriander and fenugreek genotypes

Variety	Coriander		Variety	Fenugreek	
	Cryo Ground (%)	Conventional Ground (%)		Cryo Ground (%)	Conventional Ground (%)
Sudha	18.53	9.50	RMt 1	5.63	4.6
RCr 436	18.88	12.80	RMt 305	5.95	4.8
Mean	18.705	11.15	Mean	5.79	4.7
SD (±)	0.247	2.333	SD (±)	0.226	0.141

Table 2. Total Phenolic content (ppm GAE 1000^{-1} ppm) and total flavonoids content (ppm QE $^{-1}$1000 ppm) in methanol crude extract from conventional and cryogenically grounded seeds of coriander and fenugreek genotypes

Variety	Coriander				Variety	Fenugreek			
	Cryo Ground		Conventional Ground			Cryo Ground		Conventional Ground	
	TPC	TFC	TPC	TFC		TPC	TFC	TPC	TFC
Sudha	63.61	20.22	19.46	13.94	RMt 1	5.63	75.722	4.6	67.377
RCr 436	87.08	16.70	62.39	15.38	RMt 305	5.95	83.544	4.8	71.028
Mean	73.345	18.46	40.925	14.66	Mean	5.79	79.633	4.7	69.202
SD (±)	16.595	2.489	30.356	.018	SD (±)	0.226	5.530	0.141	2.581

Table 3. Antioxidant Content and DPPH Scavenging % in methanol crude extract from conventional and cryogenically grounded seeds of coriander genotypes

Variety	Coriander				Variety	Fenugreek			
	Cryo Ground		Conventional Ground			Cryo Ground		Conventional Ground	
	Antioxidant Content	DPPH Scavenging %	Antioxidant Content	DPPH Scavenging %		Antioxidant Content	DPPH Scavenging %	Antioxidant Content	DPPH Scavenging %
Sudha	10.21	81.83	4.92	40.22	RMt 1	9.32	74.86	3.29	27.22
RCr 436	8.72	70.07	6.47	52.44	RMt 305	11.08	88.70	8.95	71.92
Mean	9.46	75.95	5.695	46.33	Mean	10.20	81.78	6.12	49.57
SD (±)	1.053	8.315	1.096	8.640	SD (±)	10.14	9.78	4.00	31.60

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