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Qualitative phytochemical and cluster analysis of genotypic extracts of coriander leaves and seeds from Tarai and Kumaun regions of Uttarakhand, Himalayan state of India

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Abstract
The aim of the present work is to study and compare the genotypic variation and similarities that exist between the Coriander leaves and seeds collected from Tarai and Kumaun region of Uttarakhand state of India. The comparative studies of preliminary phytochemical investigation using qualitative test is been performed on methanolic extracts of seventeen genotypes of Coriander leaves as well as seeds. The phytochemical screening revealed the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, fixed oils and fats, phenols, tannins, flavonoids, protein and amino acids while absence of resins and tri terpenes in both leaves and seeds genotypes. The type of phytoconstituents of leaves and seeds of different genotypes were not similar. A statistical technique, cluster analysis has been performed in order to deduce consequential knowledge that displays variation in the data meticulously. This dissimilitude in the makeup of different phytoconstituents might be used for various applications such as health supplement and pharmaceutical benefits.

Keywords: Coriander, leaves, seeds, genotypes, extracts, spices

Introduction
Coriander is an annual herb belonging to family Apiaceae, originating from the Mediterranean countries [1]. India is the biggest producer, consumer and exporter of Coriander in the world with an annual production averaging around 3 lakh tonnes. The whole plant and especially the unripe fruit, is characterized by a strong disagreeable odour, whence the name Coriander giving characteristic aroma when rubbed [2]. All parts of the plant are edible but the fresh leaves and the dried seeds are the most common parts used in cooking medicine. The seeds are mainly responsible for the medical use of Coriander and have been used as a drug for indigestion, against worms, rheumatism and pain in the joints [3].

Increased use of herbs and spices as flavourings and seasonings in food products is a major trend worldwide [4]. Most of the herbs and spices used by humans in food commodities also yield useful medicinal compounds [5, 6]. Spices play a significant role in our national economy and constitute an important group of agricultural commodities. There is increasing interest and research in the health-promoting and protective properties of herbs and spices [7-9]. Spices have a diverse array of natural phytochemicals that have complementary and overlapping actions [10].

Phytochemicals, which are biologically active naturally occurring chemical compounds in plants are recommended in cystitis, urinary tract infection, urethritis, urticaria, rash, burns, vomiting, indigestion, sore throat, nosebleed, cough, allergies, hay fever, dizziness and amoebic dysentery [11-13]. Plants, including herbs and spices, have many phytochemicals which are a potential source of natural antioxidant, e.g., phenolic diterpenes, flavonoids, alkaloids, tannins and phenolic acids [14-16]. Recently, there have been tremendous efforts to find and implement cost-effective, safe, and potent phytochemicals which act as natural antioxidants from various plant sources [17]. In present era, the emerging interest in plant derived drugs is mainly due to the current widespread belief that “green medicine” is safe and more dependable than the synthetic drugs, many of which are costly and have adverse side effects [18].

The current investigation was planned to assess preliminary phytochemical screening among seventeen Coriander leaves and seeds cultivars on the basis of genetic variability for further utilization in future.

Materials and methods

Source of plant material

Seeds and fresh leaves of different genotypes of Coriander were collected from Vegetable Research Centre (V.R.C) of G.B.P.U.A & T, Pantnagar and from Kumaun hills, Uttarakhand. Out of the total seventeen genotypes collected for experimental analysis of both leaves and seeds, fifteen genotypic varieties were developed in Pantnagar Tarai area *viz.* Pant haritima, PD-21, PD-51, UD-643, UD-684, UD-699, UD-704, UD-711, UD-716, UD-720, UD-721, UD-722, UD-725, UD-727, UD-728 and two were collected from Kumaun Region *viz.* PD 52, Pithoragarh Region and PD 53, Berinag Region of Uttarakhand State.

Site of experimental study

The present study was carried out in Department of Chemistry, College of Basic Sciences and Humanities and vegetable Research Center (V.R.C.), G.B. Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar (Uttarakhand). Regular visits were made to collect the fresh foliage (leaves and stems) and dried seeds of Coriander and care were taken to select healthy plants. The nomenclature and genotypic authenticity of all the varieties were identified by Dr. Dhirendra Singh, Professor and Joint Director, V.R.C., Department of Vegetable Science.

Chemicals and glasswares

The solvents and chemicals used were of gravimetric reagent grade and analytical reagent grade and were obtained from Hi-media and E. Merck. The glassware's used during the study were of Borosil make.

Preparation of extracts

The collected samples of Coriander leaves were washed in a running tap to remove soil and dust particles and then air dried in the laboratory for seven days. The dried samples of Coriander leaves and seeds were ground to coarse powder with a mechanical grinder. 1 gm of powdered test samples of different genotypes of leaves and seeds were soaked for 7 days in 10 mL of methanol and stored at 4°C in a dry, clean container with lid for further analysis. Working standard of desired concentration of methanolic extracts were then prepared each time from the stock to conduct the experiment.

Preliminary phytochemical screening

All the extracts of Coriander leaves and seeds genotypes were tested chemically for the detection of various metabolites *viz;* alkaloids, carbohydrates, glycosides, saponins, phytosterols, fixed oils and fats, resins, phenols, tannins, flavonoids, protein and amino acids and tri terpenes by using standard reported protocols [19].

1. Detection of alkaloids

Following methods were used to test the presence of alkaloids:

a) Wagner's Test: Extracts were treated with Wagner's reagent (Iodine in potassium iodide). The formation of a brown / reddish brown precipitate indicated the presence of an alkaloid.
b) Dragendroff's test: Extracts were treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of a red precipitate indicated the presence of an alkaloid.
c) Hager's test: Extracts were treated with Hager's reagent (saturated picric acid solution). Formation of a yellow colored precipitate indicated the presence of an alkaloid.

2. Detection of carbohydrates

The extracts were used to test for the presence of carbohydrates by different tests as follows:

a) Molisch's test: Extracts were treated with few drops of alcoholic α-naphthol solution in a test tube and 2 ml. of conc. sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicated the presence of carbohydrates.
b) Fehling's test: Extracts were hydrolysed with dil. HCl neutralized with alkali and heated with Fehling solution A and Fehling solutions B. Formation of a red precipitate indicated the presence of reducing sugars.

3. Detection of glycosides

Extracts were subjected to test for glycosides by different methods as under:

a) Modified Borntrager's Test: Different extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicated the presence of anthranol glycosides.
b) Legal's Test: The extracts were treated with sodium nitroprusside in pyridine and methanolic alkali. Formation of pink to blood red colour indicated the presence of cardiac glycosides.
c) Keller-Killiani test: The extracts were treated with 1 ml glacial acetic acid, $FeCl_3$ and conc. H_2SO_4 successively in mix. Formation of green-blue colour indicated the presence of cardiac glycosides.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water and shaken in a graduated cylinder for some time. Formation of 1 cm layer of foam indicated the presence of saponins.
b) Foam Test: Small amount of extract was shaken with little quantity of water. Persistence of foam for ten minutes it indicated the presence of saponins.

5. Detection of phytosterols

The phytosterols were tested by using following well known tests:

a) Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken and allowed to stand. The appearance of golden yellow color indicated the presence of triterpenes.
b) Tshugajeu test: Extracts were treated with chloroform and filtered. Excess of acetyl chloride and a pinch of zinc chloride were added, kept aside for some time till the reaction was completed and then warmed on water bath. The appearance of blurred red colour indicated the presence of triterpenes.

6. Detection of fixed oils & fats

Following tests were performed to detect the fixed oil and fats in extracts.

a) Stain Test: Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicated the presence of fixed oil.

7. Detection of resins

Following were the test for the detection of resins in different extracts.

a) Acetone-water Test: Extracts were treated with acetone. Small amount of water was added and shaken. The appearance of turbidity indicated the presence of resins.

8. Detection of phenols

To detect phenol in extracts ferric chloride test was performed as under.

a) Ferric Chloride Test: Extracts were treated with a few drops of ferric chloride solution. Formation of bluish black color indicated the presence of phenols.

9. Detection of tannins

The tannins were detected by gelatin test.

a) Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicated the presence of tannins.

10. Detection of flavonoids

Following three tests were performed for the detection of flavonoids in the extracts:

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on the addition of dilute acid, indicated the presence of flavonoids.
b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of a yellow colored precipitate indicated the presence of flavonoids.
c) Shinoda Test: To the alcoholic solution of extracts, a few fragments of magnesium ribbon and concentrated HCl were added. The appearance of magenta color after a few minutes indicated the presence of flavonoids.

11. Detection of proteins and amino acids

a) Xanthoproteic Test: The extracts were treated with a few drops of concentrated Nitric acid solution. Formation of yellow color indicated the presence of proteins.
b) Ninhydrin Test: To the extract, 0.25% ninhydrin reagent were added and boiled for a few minutes. Formation of blue color indicated the presence of amino acids.
c) Biuret Test: The extracts were treated with 1 ml of 10% sodium hydroxide solution and heated. To this a drop of 0.7% copper sulphate solution was added. Formation of purplish violet color indicated the presence of proteins.

12. Detection of triterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with a few drops of copper acetate solution. Formation of emerald green color indicated the presence of triterpenes.

Results and discussions

Methanolic extracts are subjected to screening for phytoconstituents assessment in order to extract the maximum components present in the Coriander leaves and seeds. Qualitative phytochemicals screening of Coriander leaves and seeds revealed some differences in the constituents of the different genotypes. All these phytochemicals are reported to possess anti-oxidant properties and various pharmacological actions. The type of phytoconstituents of the leaves and seeds in some genotypes were not similar indicating the variation, whilst, some genotypes showed the presence of many phytochemicals somewhere common in both leaves and seeds. The phytochemical screening of methanolic extracts revealed the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, fixed oils and fats, phenols, tannins, flavonoids, protein and amino acids while absence of resins and tri terpenes in both leaves and seeds genotypes on the basis of colour change tests. It was observed that the type of phytoconstituents of the leaves and seeds of different genotypes were not similar. Table 1 and table 2 represent the presence and absence of phytochemicals in leaves and seeds respectively.

The findings are in agreement with Thangavel *et al.*, who studied preliminary phytochemicals in Coriander seeds extracts in different solvents. It was evident from their study that methanolic extracts of Coriander seeds showed the better results for the presence of alkaloids, protein and amino acids, glycosides, flavonoids, tannins and phenolic compounds and saponins [20]. Our results were also in agreement with Ishikawa *et al.*, who studied the presence of phyto compounds in Coriander seeds [21]. Dharmalingam and Nazni studied the phytochemical evaluation of Coriander flowers extracted with methanol based on the presence or absence of colour changes. Methanolic extracts of Coriander flower contained alkaloids, flavonoids, steroids, reducing sugar and glycosides, saponins and tannins [22].

The numerous properties of these phytochemicals have made them more charismatic, as they can modulate various aspects of disease like lipid peroxidation involved in carcinogenesis, atherogenesis, thrombosis, hepatotoxicity and a variety of disease conditions [23]. The potential of Coriander leaves and seeds genotypes as a promising and rich source of natural source of phytochemicals lead us to propose Coriander leaf and seed suitable for application in nutritional and pharmaceutical field.

Cluster analysis

Cluster analysis was performed on the phytoconstituents in various genotypes of Coriander leaves and seeds using software The Unscrambler X 10.5 applying Ward's method with Square Euclidean distance. The dendrogram of cluster analysis is presented in Fig.1 where S and L denotes seeds and leaves genotypes respectively. Based on the phytoconstituents similarities obtained in several preliminary screening, various genotypes of Coriander leaves and seeds were grouped into five clusters as:

Group 1: S-PD 21, L-PD 21, S-Pant haritima, L- Pant haritima.

Group 2: S-PD 53, L-PD 53, S-PD 52, L-PD 52.

Group 3: S-UD 727, S-UD 725, L-UD 727, L-UD 725, S-UD 716, L-UD 716, S-UD 720, S-UD 699, L-UD 720, L-UD 699, S-UD 643, L-UD 643.

Group 4: S-UD 722, L-UD 722, S-UD 721, L-UD 721, S-UD 728, S-UD 711, S-UD 684, L-UD 728, L-UD 711, L-UD 684, S-PD 51, L-PD 51.

Group 5: S-UD 704, L-UD 704.

Table 1: Phytochemical specifications of Coriander leaves

Phytoconstituent	Test	Pant haritima	PD 21	PD 51	PD 52	PD 53	UD 643	UD 684	UD 699	UD 704	UD 711	UD 716	UD 720	UD 721	UD 722	UD 725	UD 727	UD 728
Alkaloids	Mayer's Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Wagner's Test	-	-	+	+	+	-	+	-	-	+	+	-	+	-	+	+	+
	Dragendroff's test	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	Hager's test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carbohydrates	Molisch's test	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Benedict's test	-	-	+	+	-	+	+	-	+	+	-	-	+	+	-	-	+
	Fehling's test	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycosides	Modified Borntrager's Test	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
	Legal's Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Keller-Killiani test	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	Froth Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Foam Test	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+
Phytosterols	Salkowski's Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Liebermann Burchard's Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Tshugajeu test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fats & Oils	Stain Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Resins	Acetone-water Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenols	Ferric Chloride Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	Gelatin Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	Alkaline Reagent Test	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+
	Lead acetate Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Shinoda Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Proteins & Aminoacids	Xanthoproteic Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Ninhydrin Test	+	+	-	+	+	+	-	+	-	-	+	+	+	-	+	+	-
	Biuret Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Triterpenes	Copper acetate Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ = presence, - =absence

Table 2: Phytochemical specifications of Coriander seeds

Phytoconstituent	Test	Pant haritima	PD 21	PD 51	PD 52	PD 53	UD 643	UD 684	UD 699	UD 704	UD 711	UD 716	UD 720	UD 721	UD 722	UD 725	UD 727	UD 728
Alkaloids	Mayer's Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Wagner's Test	-	-	+	+	+	-	+	-	-	+	+	-	+	-	+	+	+
	Dragendroff's test	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	Hager's test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carbohydrates	Molisch's test	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Benedict's test	-	-	+	+	-	+	+	-	+	+	-	-	+	+	-	-	+
	Fehling's test	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycosides	Modified Borntrager's Test	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
	Legal's Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Keller-Killiani test	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	Froth Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Foam Test	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+
Phytosterols	Salkowski's Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Liebermann Burchard's Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Tshugajeu test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fats & Oils	Stain Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Resins	Acetone-water Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenols	Ferric Chloride	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

	Test																	
Tannins	Gelatin Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	Alkaline Reagent Test	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+
	Lead acetate Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Shinoda Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Proteins & Aminoacids	Xanthoproteic Test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Ninhydrin Test	+	+	-	+	+	+	-	+	-	-	+	+	+	-	+	+	-
	Biuret Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Triterpenes	Copper acetate Test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ = presence, - =absence

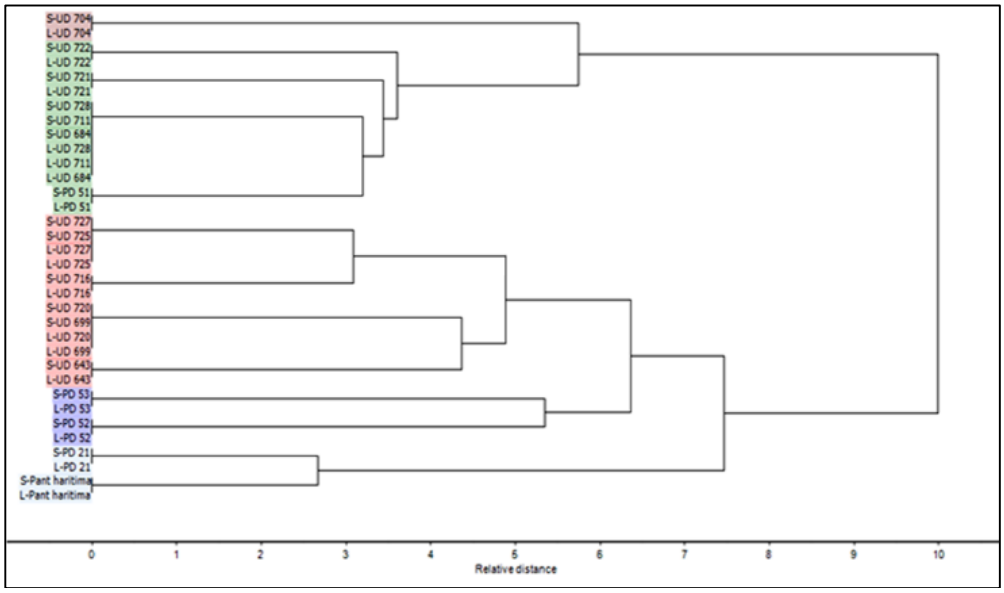

Fig 1: Dendrogram of various genotypes of Coriander leaves and seeds grouped into five clusters

Conclusion

Environment plays a dominant role and has an influential effect to enhance the yield of the Coriander herb, seed and oil. To the best of our knowledge, no report exists regarding the preliminary phytochemical analysis of Coriander leaves and seeds genotypes developed in Tarai and Kumaun regions of Uttarakhand. These genotypic variations may be attributed to the environmental variations that cause change in the content and composition, quality and yield of Coriander that provides rich source of phytochemicals. From the present study, it is concluded that the leaves and seeds of Coriander genotypes are the good sources of phytochemicals, the non-nutrient plant chemicals that contain protective, disease preventing compounds and surely helpful in treating the disease associated with oxidative stress. Clustering of all the genotypes into five groups using cluster analysis provide an important exploratory technique to investigate the similarities that exists between Coriander leaves and seeds genotypes on the basis of preliminary screening. This genetic variability that exists between the genotypes of Coriander may be used for herbal medicine and useful for food and drugs. Further studies have to be carried out to reveal the activities of these phytoconstituents present in this plant material.

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