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Antibacterial Efficacy and Cluster Analysis of Genotypic Extracts of Coriander Leaves and Seeds against Human Pathogenic Bacterial Strains

Ankita Verma*, Deepali Agarwal, Jyotsna Dhanik, Neelam Arya and Viveka Nand

Department of Chemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar – 263145, Uttarakhand, India

**Corresponding author*

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A B S T R A C T

The present study was undertaken to evaluate the *in vitro* antibacterial activity of seventeen genotypes of Coriander leaves and seeds collected from Tarai and Kumaun regions of Uttarakhand state of India. The methanolic extracts of leaves and seeds genotypes were screened against four human pathogenic bacterial strains; *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* at four different concentrations. All the methanolic extracts of leaves and seeds had inhibitory effect against tested bacteria *E. coli*, *S. typhi* and *B. subtilisi*, while no antibacterial activity was observed against *P. aeruginosa* for both leaves and seeds extracts. The study also evaluates the genotypic variation and similarities that exist between the Coriander leaves and seeds on the basis of its antibacterial potency through a statistical approach i.e. cluster analysis, regarding genetic makeup of Coriander cultivars so that future investigation may be carried out to develop a new variety for further applications in pharmacology.

Introduction

Spices are products from plant leaves, seeds, fruits, flowers, roots or bark, that are added to food commodities to enhance flavour, colour or to minimize the rate of rancidity and as preservatives that suppress microbial activities (Teye *et al.*, 2013). The beneficial characteristics of natural plant products could increase food safety and shelf life of processed food products (Dhanik *et al.*, 2017). The benefits of these herbs and spices are not hidden and it is only in recent years that modern science has started paying attention to the properties of spices (Chaudhry and Tariq, 2006). These plants then emerged as compounds with potentially significant therapeutic application against human pathogens, including bacteria, fungi or virus (Horvath *et al.*, 2002; Jantan *et al.*, 2003; Khan *et al.*, 2003; Perez, 2003).

These traditional medicinal plants contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Reynolds, 1996). The antibacterial activity of various spices and medicinal plants has been known for years. Plant oils and extracts have been used for a wide variety of purposes for many thousands of years (Hashim *et al.*, 2010). The use of these plants containing natural compounds could open up the possibility of using them as novel antimicrobials (Dhanik *et al.,* 2017).

One of the widely used spices having tremendous medicinal properties is Coriander. Coriander, also called as ‘‘cilantro”, is an annual herbaceous plant of family Apiaceae originating from Middle Eastern and Mediterranean regions cultivated for its aromatic and medicinal use (Mildner *et al.*, 2009). It is considered both as an herb and a spice since both of its leaves and seeds are used as a seasoning condiment. Researches on potential botanical extracts which are safe and environmentally benign are urgently needed. The medicinal properties of Coriander may be attributed due to the presence of various phytoconstituents present in its leaves and seeds. In our previous work (Verma *et al.*, 2018), we have investigated that the type of phytoconstituents of leaves and seeds of different genotypes are not similar. The use of this magical plant is not only limited to household health benefits rather it is also used in agricultural field for control of insect and pests due to the presence of plant secondary constituents, that plays a prominent role with different mode of action against insects. Genotypic extracts of Coriander leaves and seeds were also tested as antifeedants against insect *S. litura* (Verma *et al.,* 2015). Coriander is also used in detox diet as it helps to remove toxic mineral residue such as mercury and lead, and excrete them in the urine or faeces (Kansal *et al.,* 2011). Coriander is considered an important herb due to its extensive use as medicine for curing capabilities against many diseases due to presence of active ingredients and components in its leaves and fruit (Kubo *et al.,* 2004).

The mountainous state of Uttarakhand, India, is endowed with a rich variety of medicinal plants, many of which are in great demand in the domestic and export markets (Government of India, 2000). Because of a variety of agro-climatic niches, the state has tremendous potential to emerge as a regular supplier of medicinal and aromatic plants (Samant *et al.*, 1998). Uttarakhand State has a rich heritage of traditional medicine system that acquires a dominant role in pharmacy and agronomy production. Pharmacology, phytochemistry and biological activities of some medicinal plant of Uttarakhand have been reported earlier by Dhanik *et al.*, (2017).

The aim of our study is to investigate the antibacterial activity of different genotypic extracts of both seeds and leaves of Coriander cultivars against four human pathogenic bacterial strains. In addition, cluster analysis was applied to achieve the knowledge about the genetic makeup of Coriander cultivars and to develop a new variety of Coriander for future pharmacological applications.

Materials and Methods

Plant material

Seventeen different genotypes of Coriander were developed and collected from Vegetable Research Centre (V.R.C) of G.B.P.U.A & T, Pantnagar and from Kumaun hills, Uttarakhand, India. Out of the total seventeen genotypes collected for experimental analysis, 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.

Preparation of plant extracts

The collected samples of Coriander leaves were washed in a running tap to remove soil and dust particles and then shade dried in the laboratory for seven days. The dried samples of Coriander leaves and seeds were pulverized into fine powder with a mechanical grinder. The material was extracted by successive soaking for 72 hours in methanol and stored in refrigerator at 4°C in a dry, clean amber coloured bottle for further analysis. Working standards of desired concentration 250, 500, 750 and 1000 µg/mL of methanolic extracts were then prepared each time from the stock to conduct the experiment.

Sources of test organisms

The antibacterial screening of methanolic leaves and seeds extracts of Coriander genotypes was evaluated against four pathogenic bacterial strains; *Escherichia coli, Salmonella typhi, Bacillus subtilis, Pseudomonas aeruginosa.* The bacterial strains used for antibacterial study was isolated from different meat samples in Department of Veterinary and Public Health, Pantnagar (India). Antibacterial screening of the extracts against these bacteria was done by Disc-diffusion method as reported by Singh *et al.,* (2005) with slight modification and was measured by zone of inhibition.

Preparation of bacterial inoculums

For the preparation of bacterial inoculums, Luria Bertani broth (Hi-media) for *Escherichia coli*, buffered peptone water (Hi-media) for *Salmonella typhi*, nutrient broth for *Bacillus subtilis* and *Pseudomonas aeruginosa* were weighed and poured in distilled water as per manufacturer's instructions. The test tubes containing culture media was sterilized in an autoclave at 120ºC and 15-20 lbs for half an hour. Bacterial colonies were inoculated in test tubes in above prepared broths. The test tubes containing bacterial colonies were incubated for 24 hr in incubator. Next day cultures showed a marked turbidity in the tubes and were used to conduct further experiment.

Preparation of agar plates

Difco nutrient agar (1.5%) was used for the preparation of plate media. The media was prepared in distilled water, autoclaved and gently cooled. Thereafter, the prepared media was poured in petri plates (dia 9 cm) in laminar flow and kept undisturbed as such till it got solidified. After solidification, these petri plates were incubated at 37^{o}C overnight for sterile testing.

Screening of antibacterial activity of methanolic extracts of coriander genotypes

The standard disc diffusion method was used for the antibacterial analysis (Bauer *et al.*, 1966; NCCLS, 2000). Bacterial inoculums 100 µL was added to the agar plates and uniformly spread over the surface using spreader (Hi-media).

Placement of the plates

Sterilized disc of 5 mm diameter soaked in different methanolic concentrations of extracts (250, 500, 750 and 1000 µg/mL) were placed on the inoculated plate. These plates were incubated at 37^{o}C overnight to observe the zone of inhibitions around the disc, which were compared with the zone of inhibitions formed by the standard antibiotic, Gentamicine. The sterile paper discs impregnated with methanol served as negative control. These inoculated plates were firmly closed with the maximum possible aseptic precautions.

Recording of observations

After incubation, relative susceptibility of each organism was determined by a clear zone of inhibition of growth around the disc impregnated with the extracts as well as the antibiotic. Zone of inhibition (mm) was measured with the help of scale.

Statistical analysis

Cluster analysis was performed using the software The Unscrambler X 10.5 applying Ward's method with Square Euclidean distance.

Results and Discussion

The antibacterial screening of various concentrations of methanolic extracts of Coriander leaves and seeds genotypes against four human pathogenic bacterial strains; *Escherichia coli, Salmonella typhi, Bacillus subtilis and Pseudomonas aeruginosa* was performed by Disc-diffusion method and antibacterial activity was measured as zone of inhibition.

All the methanolic extracts of leaves and seeds had inhibitory effect against tested bacteria; *E. coli, S. typhi and B. subtilis* while no antibacterial activity was observed with *P. aeruginosa* for both leaves and seeds extracts.

Methanolic extract of PD-51 leaves and Pant haritima seeds exhibited maximum zone of inhibition for all the bacterial strains. Leaves extracts of UD-684, UD-704, UD-711, UD-716, UD-721 and PD-52 were found to be ineffective against *B. subtilis.* Similarly, seeds extracts of UD-720 and UD-727 showed no activity against *S. typhi.*

The zone of inhibition for Coriander leaves and seeds genotypes as recorded for different bacteria are presented in table 1 and 2, figure 1 and 2. The inhibitory effect of Coriander genotypes was comparatively less than that of standard antibiotic, Gentamicin. Ali *et al.,* (2008) reported that the antimicrobial efficiency of Coriander leaf extracts is due to the presence of flavonoids and terpenoids. The essential oil and various extracts from Coriander have been shown to possess antibacterial, antidiabetic, anticancerous, antimutagenic, antioxidant and free radical scavenging activities (Sreelatha *et al.,* 2009; Zoubiri and Baaliouamer, 2010). It is also reported that Coriander essential oil has powerful antibacterial activity against gram positive (*Staphylococcus aureus* and *Bacillus* spp.) and gram negative (*Escherichia coli, Salmonella typhi, Klebsiella pneumonia* and *Proteus mirabilis*) bacteria (Cantore *et al.,* 2004; Matasyoh *et al.,* 2009). From the present study, we conclude that methanolic extracts of Coriander leaves and seeds have pronounced antibacterial potency and respond differently to different bacteria with variable zone of inhibition. Elgayyae *et al.,* (2001) reported that the antibacterial effect of Coriander appeared to be highly variable depending on plants conditions and species.

Cluster analysis

Cluster analysis applied on the entire data including all the concentrations of methanolic extracts of leaves and seeds of Coriander genotypes, gave respective dendrograms presented in figure 3 and 4, where prefix L and S denotes leaves and seeds genotypes respectively. Based on the antibacterial efficacy of Coriander genotypes as screened against bacterial strains various genotypes of Coriander leaves and seeds were grouped into five clusters each as:

Clustering in leaves genotypes

Group 1: L-UD-699, L-UD-725, L-PD-53

Group 2: L-UD-722, L-UD-728, L-UD-720, L-PD-51

Group 3: L-UD-643, L-Pant haritima

Group 4: L-UD-704, L-UD 684, L-PD-21

Group 5: L-UD-721, L-UD-716, L-UD-711, L-UD-727, L-PD-52

Table.1 Effect of Coriander leaves genotypes on human pathogenic bacteria by disc diffusion method

Leaves Genotypes	Zone of inhibition (mm)															
	Escherichia coli				***Salmonella typhi***				***Bacillus subtilis***				***Pseudomonas aeruginosa***			
	Conc.(ppm)				**Conc. (ppm)**				**Conc. (ppm)**				**Conc. (ppm)**			
	250	**500**	**750**	**1000**	**250**	**500**	**750**	**1000**	**250**	**500**	**750**	**1000**	**250**	**500**	**750**	**1000**
Pant haritima	-	12	12	14	10	12	-	-	12	12	-	12	-	-	-	-
PD-21	-	-	-	16	-	10	12	12	-	-	-	12	-	-	-	-
PD-51	10	16	18	20	-	-	-	14	-	12	12	14	-	-	-	-
PD-52	10	10	12	12	-	-	10	12	-	-	-	-	-	-	-	-
PD-53	10	10	10	-	-	10	10	10	12	-	10	14	-	-	-	-
UD-643	-	16	16	-	12	10	-	-	10	10	12	16	-	-	-	-
UD-684	-	-	-	-	-	10	12	12	-	-	-	-	-	-	-	-
UD-699	10	10	10	14	-	10	14	-	12	12	14	-	-	-	-	-
UD-704	-	-	-	12	-	12	10	-	-	-	-	-	-	-	-	-
UD-711	14	12	14	16	10	-	-	10	-	-	-	-	-	-	-	-
UD-716	10	10	10	12	16	12	12	10	-	-	-	-	-	-	-	-
UD-720	-	14	14	20	-	8	10	12	-	10	10	12	-	-	-	-
UD-721	12	10	10	-	-	10	10	16	-	-	-	-	-	-	-	-
UD-722	-	10	10	10	-	12	12	12	-	-	14	14	-	-	-	-
UD-725	10	12	12	14	-	10	12	12	10	-	-	12	-	-	-	-
UD-727	-	10	12	16	-	-	-	14	-	-	-	-	-	-	-	-
UD-728	-	12	12	12	-	8	10	12	12	12	14	16	-	-	-	-
Gentamicine	24				24				24				20			
Control (Methanol)	-				-				-				-			

(-) sign indicates no inhibition

Table.2 Effect of coriander seeds genotypes on human pathogenic bacteria by disc diffusion method

Seeds Genotypes	Zone of inhibition (mm)															
	Escherichia coli				*Salmonella typhi*				*Bacillus subtilis*				*Pseudomonas aeruginosa*			
	Conc.(ppm)				**Conc. (ppm)**				**Conc. (ppm)**				**Conc. (ppm)**			
	250	**500**	**750**	**1000**	**250**	**500**	**750**	**1000**	**250**	**500**	**750**	**1000**	**250**	**500**	**750**	**1000**
Pant haritima	-	-		16	12	12	16	16	-	10	12	14	-	-	-	-
PD-21	10	12	12	14	-	12	12	14	-	10	10	12	-	-	-	-
PD-51	-	10	12	14	10	10	10	12	10	12	12	14	-	-	-	-
PD-52	10	12	14	16	-	-	-	10	12	12	12	14	-	-	-	-
PD-53	10	14	14	16	-	10	10	12	-	12	14	14	-	-	-	-
UD-643	-	12	14	16	10	12	12	14	-	-	-	10	-	-	-	-
UD-684	-	-	-	-	-	12	14	14	-	-	-	12	-	-	-	-
UD-699	10	12	12	14	-	10	12	14	10	10	12	12	-	-	-	-
UD-704	-	-	-	12	-	10	10	12	-	10	10	12	-	-	-	-
UD-711	-	14	14	16	12	12	14	16	12	12	14	16	-	-	-	-
UD-716	-	12	12	14	10	12	14	14	10	10	12	12	-	-	-	-
UD-720	-	14	14	16	-	-	-	-	-	10	10	12	-	-	-	-
UD-721	10	12	12	14	-	12	16	16	-	12	14	14	-	-	-	-
UD-722	10	12	12	12	-	10	12	12	12	12	12	14	-	-	-	-
UD-725	-	10	12	14	-	10	10	10	10	10	12	14	-	-	-	--
UD-727	-	12	14	14	-	-	-	-	-	-	-	12	-	-	-	-
UD-728	-	14	14	16	-	10	12	12	-	-	-	14	-	-	-	-
Gentamicine	24				24				24				20			
Control (Methanol)	-				-				-				-			

(-) sign indicates no inhibition

Fig.1 Zone of inhibition in different bacterial strains by coriander leaves genotypes

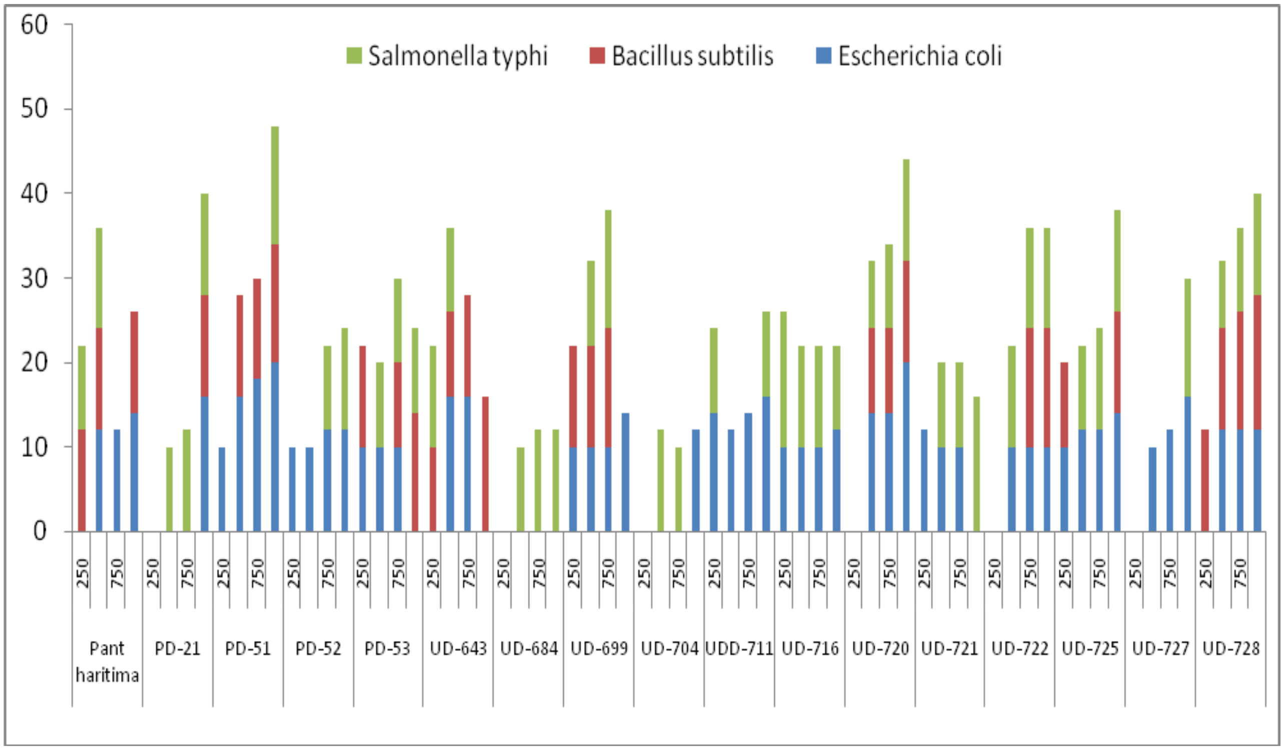


Fig.2 Zone of inhibition in different bacterial strains by coriander seeds genotypes

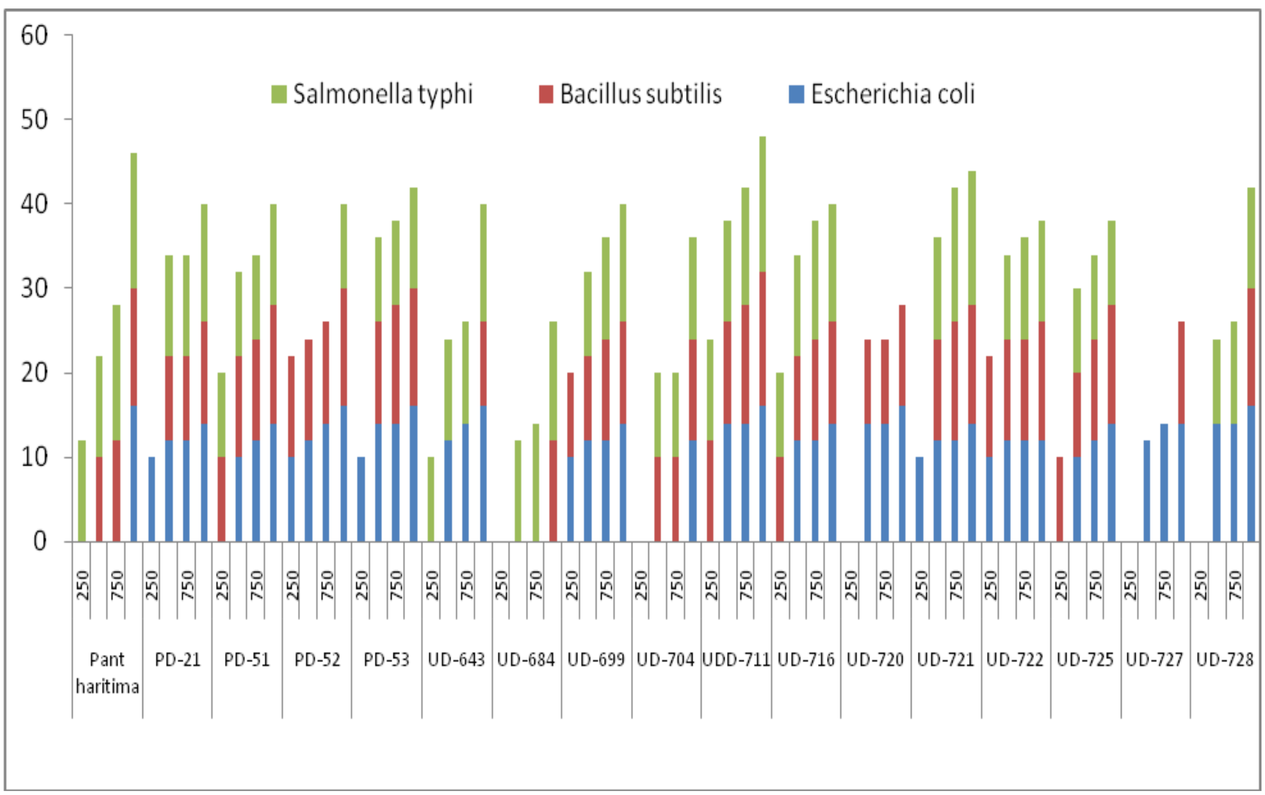


Fig.3 Dendrogram of various genotypes of coriander leaves grouped into five clusters

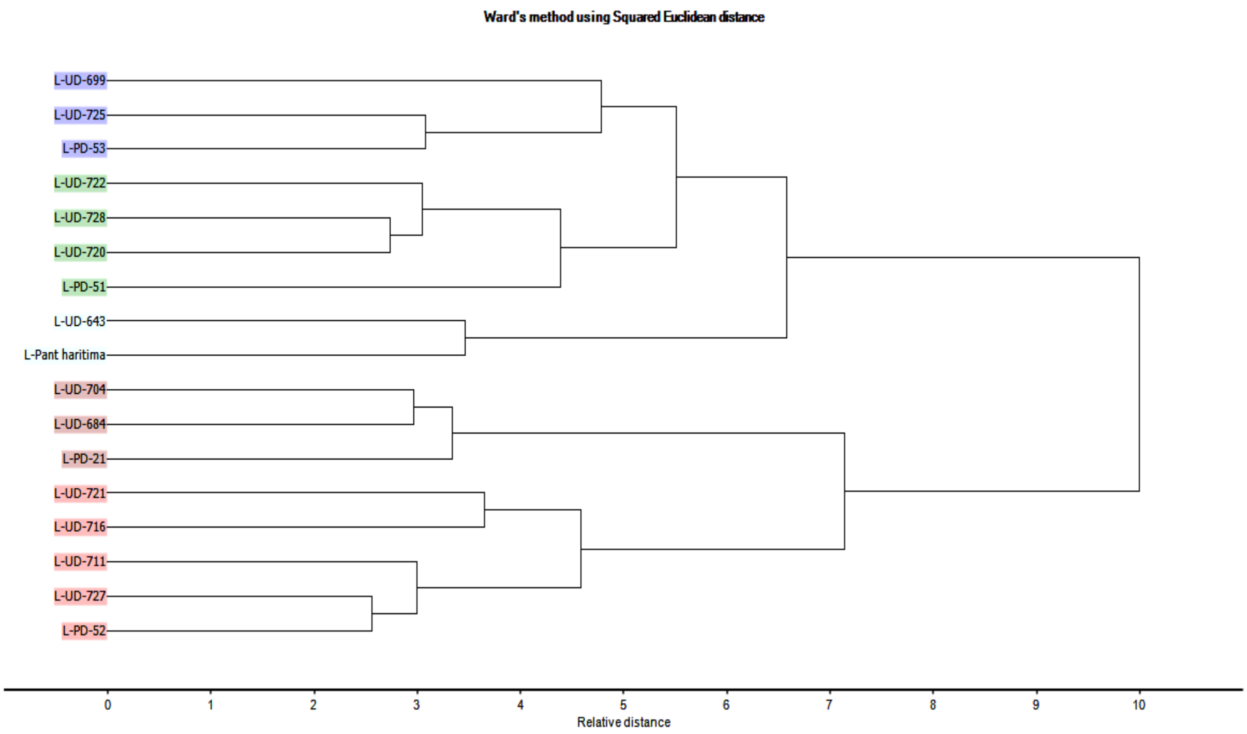
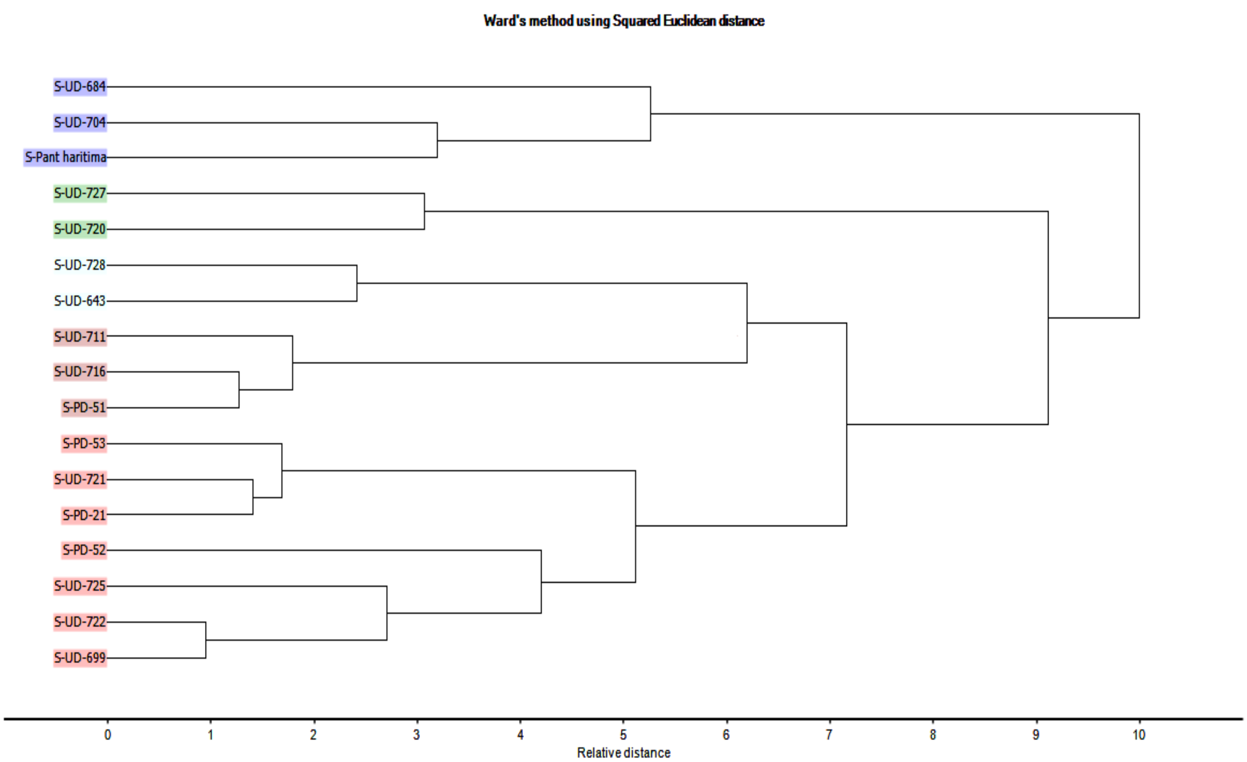


Fig.4 Dendrogram of various genotypes of coriander seeds grouped into five clusters



Clustering in seeds genotypes

Group 1: S-UD-684, S-UD-704, S-Pant haritima

Group 2: S-UD-727, S-UD-720

Group 3: S-UD-728, S-UD-643

Group 4: S-UD-711, S-UD-716, S-PD-51

Group 5: S-PD-53, S-UD-721, S-PD-21, S-PD-52, S-UD-725, S-UD-722, S-UD-699

This scientific and statistical information provides an important platform for the development of effective natural medicines by developing clusters of Coriander genotypes which can serve as a potential natural antibacterial plant extract for bacteria's which are threat to human health.

To the best of our knowledge, this is the first report on antibacterial efficacy of Coriander leaves and seeds genotypes developed and collected from Tarai and Kumaun region of Uttarakhand state and present study results suggest that this new investigation could be first step to develop a new variety of Coriander leaves and seeds for future applications in pharmacology. Cluster analysis provides five clusters of respective genotypes of Coriander leaves and seeds depending on their potency of antibacterial activity irrespective of concentration. This study provides evidence for further statistical approach to identify the active components responsible for the plant biological activity.

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