Characterization of Bioactive Compounds and Antioxidant Activity ~~in~~ among Genetically Different Genotypes of Chilli (*Capsicum annum* L.) ~~Under North Western Himalayas Region of Jammu and Kashmir, India~~

ABSTRACT

This ~~current study was designed to~~ evaluated the antioxidant capacity and total phenolic contents from forty-five genotypes of chilli ~~collected from different states of India representing different agro ecological regions~~. The antioxidant property was assayed by scavenging abilities using diphenyl-2-picrylhydrazyl (DPPH), azinobisethylbenzothiazoline-6-sulphonic acid (ABTS), assay of ferric reducing antioxidant power (FRAP), and determining total phenolics (TP) and total flavonoids (TF) contents. There was a significant variation in the total phenolic content (17.38–131.5mg GAE/g dry weight), total flavonoid (14.07–56.15 mg quercetin/g dry weight), DPPH (0.55–5.60 mM AAE/g dry weight), ABTS (16.03– 38.12 mM AAE/g dry weight) and FRAP (0.80– 6.40 mM GAE/dry weight). Three genotypes viz. IC-561635, CITH-HP-22 and IC-561731 exhibited highest values for all the antioxidant assays. Positive and~~ly~~ significant correlations ~~coefficients~~ were observed between ABTS and FRAP (INDICATE STATISTICS), TF and FRAP (INDICATE STATISTICS), TP and FRAP (INDICATE STATISTICS), TP and DPPH (INDICATE STATISTICS), and TP and TF (INDICATE STATISTICS). ~~Forty five~~ Hierarchical cluster analysis grouped the studied genotypes ~~of chilli were grouped~~ into seven clusters ~~based on the standardized squared Euclidean distance using Ward's hierarchical clustering method~~. The ~~experiment established that the~~identified genotypes of chilli are potent sources of natural antioxidants which reduce the oxidation processes in the body by protecting against reactive oxygen species.

Keywords: *Chilli, genotypes, antioxidant*

INTRODUCTION:

The Novel Coronavirus disease-2019 (COVID-19 has inflicted mayhem worldwide, claiming more than 5.5 million lives and infecting more than 323 million people [1]. The vaccine is now available against COVID-19 but still the importance of natural compounds of inhibition and remedy cannot be ignored. In this aspect, the food and dietary habit play key perspectives in deciding general wellbeing and resistance [2].

Vegetables are a good option to build resilience in the body against infection. Vegetables are importance because being low in calories are packed with vitamins, minerals, antioxidants and photochemical. Therefore, the use of natural compounds may provide an alternative prophylactic and therapeutic support along with the therapy for COVID-19. Chilli (*Capsicum annuum* L.) is highly valued as an excellent source of natural pigments and antioxidant compounds. Chilli has varied uses in diverse situations, it is used as spice, condiment, traditional medicine, vegetable or ornamental plant. Chilli

Formatted: Indent: Left: 0.3"
Formatted: Numbering: Continuous
Formatted: Font: Italic
Comment [ZT1]: What about flavonoids
Comment [ZT2]: Reconstruct
Comment [ZT3]: What about the superior genotypes for other variables, other than antioxidants?
Comment [ZT4]: Revise the statement and account for all variables mentioned in the study
Formatted: Font: Not Bold
Formatted: Font: Not Bold, Italic
Formatted: Font: Not Bold
Formatted: Indent: Left: 0"
Formatted: Indent: Left: 0.3"
Comment [ZT5]: Why capitalize these words
Comment [ZT6]: Rephrase
Comment [ZT7]: Revise please. The English is not good. Also, even the previous sentence began with 'Vegetables"????
Comment [ZT8]: Reference these sentences

is an indispensable spice in Indian cuisines owing to its pungency, colour and aroma. ~~Nutritionally, it~~The crop is a rich source of nutrients, including vitamins A, C, E and ~~,~~ thiamine, minenrals [i.e., molybdenum, manganese ~~, potassium~~and potassium]~~,~~ carotenoids, and phenolic compounds [3]. These compounds provide ~~many~~ nutritional and health benefits that include antioxidant, anti-~~inflammatory, and~~inflammatory and antimicrobial activities, reduced prevalence of obesity and type ~~-~~2 diabetes ~~and obesity~~, protection against hypercholesterolemia, and reduced prevalence of atherosclerotic cardiovascular diseases [4,5]

However, the composition and levels of specific phytochemicals with antioxidant potential present in vegetables do not essentially imitate the total antioxidant capacity, which depends on the type and concentration of phytochemicals, as well as the coactive or inhibitory interaction of molecules in the matrix. Therefore, it is imperative to study the phytochemicals present in vegetables of high importance like chilli, in order to generate information about their possible health benefits. These nutrients can be repurposed in mitigating the pathological effects induced by the SARS-CoV-2 infection. The objective of this study was to ~~carried out to~~ investigate the antioxidant properties (total phenolic content, total flavonoid content) in forty-five Chilli ~~(*Capsicum annuum* L.)~~ cultivars grown in Kashmir.

Material and method

Planting material/ samples~~:~~

~~The experimental material comprised of f~~Forty-five genetically diverse chilli genotypes ~~of chilli~~ collected from different states of India representing different agro-ecological regions were evaluated for various quantitative and quality traits at the Experimental Field, Division of Vegetable Science, SKUAST-Kashmir, Shalimar Srinagar, during Kharif season in 2021. ~~Details of g~~Genotype names and~~s along with their~~ source~~s~~ are presented in the Table-1. Seeds were removed from red ripe stage fruits of uniform physiological maturity and pericarp along with placenta were left to dry, in air oven (40 ^{0}C) for 15 days and then powdered with pestle and mortar and passed through a 100-mesh sieves and extraction was carried out with methanolic extract, the material (5 g of fruits) was extracted with 70 % ethanol (plant: solvent, 1:10, w/v) under mechanical orbital shaker at room temperature for 72 h. Extracts were filtered using a Buckner funnel and Whatman No. 1 filter paper and ethanol was allowed to dry. Each extract was suspended in methanol to make 50 mg~~/~~ ml^{-1} stock solution.

Comment [ZT9]: Please indicate quantities in brackets for each trait mentioned

Comment [ZT10]: Add two more references

Comment [ZT11]: First time seeing this in the introduction section. Please introduce the term

Formatted: Highlight

Formatted: Indent: Left: 0"

Formatted: Indent: Left: 0.3"

Comment [ZT12]: Provide experimental design and geographical conditions information

Comment [ZT13]: Remove space

Comment [ZT14]: Such a long sentence. Please reformulate

Formatted: Superscript

Formatted: Indent: Left: 0"

Table 1: ~~List~~ Names and sources **of chilli** ~~(*Capsicum annuum* L.)~~ **genotypes used in the present study.**

S. No.	~~Chilli~~ Genotype names	Source	S. No.	~~Chilli~~ Genotype names	Source
1.	LSVT-Red - 1	Gujarat	27.	IC-561627	NBPGR
2.	LSVT-Red-2	Gujarat	28.	SK-SC-1162	CITH-Srinagar
3.	LSVT-Red-3	Gujarat	29.	SKAU-078	SKUAST-K
4.	Kashmiri Long-1	SKUAST-K	30.	V0BC-0289	Orrisa
5.	IC-561652	NBPGR	31.	Jawahar Mirch	Jabalpur Madhya Pradesh
6.	IC-561614	NBPGR	32.	Guccha Mirch-1	Chamba-Himachal Pradesh
7.	IC-561610	NBPGR	33.	SK-SC-1161	CITH-Srinagar
8.	IC-561730	NBPGR	34.	Guccha Mirch-2	Chamba - Himachal-Pradesh
9.	IC-561665	NBPGR	35.	CITH-HP-17/13	CITH-Srinagar

Formatted: Indent: Left: 0.3", Line spacing: Double

10.	IC-572487	NBPGR	36.	ARCH-228	IIVR
11.	IC-561618	NBPGR	37.	SKAU-084	SKUAST K
12.	IC-561661	NBPGR	38.	G-4	Andhra Pradesh (ANGRAU)
13.	IC-561691	NBPGR	39.	CITH-HP-171/13	CITH-Srinagar
14.	Kashi Anmol	Varanasi (IIVR)	40.	CITH-HP-22	CITH-Srinagar
15.	IC-561657	NBPGR	41.	Sel-680/11	CITH-Srinagar
16.	CITH-HP-16	CITH-Srinagar	42.	CITH-HP-71/13	CITH-Srinagar
17.	IC-561731	NBPGR	43.	SKAU-089	SKUAST-K
18.	IC-561622	NBPGR	44.	CITH-HP-1154-1/13	CITH-Srinagar
19.	Sel-839-2	CITH-Srinagar	45.	SKAU-092	SKUAST-K
20.	CITH-HP-111	CITH-Srinagar	46.	SKAU-096	SKUAST-K
21.	Sel-917-111	CITH-Srinagar	47.	Goa-Sel-1	Goa
22.	CITH-HP-1154	CITH-Srinagar	48.	SKASU-111	SKUAST-K

Formatted: Indent: Left: 0.3", Line spacing: Double

23.	IC-561631	NBPGR			
24.	IC-561635	NBPGR			
25.	IC-561639	NBPGR			
26.	Pusa Sadabahar	New Delhi (IARI)			

Formatted: Indent: Left: 0.3", Line spacing: Double

Formatted: Indent: Left: 0.3", Line spacing: Double

Formatted: Indent: Left: 0.3", Line spacing: Double

Comment [ZT15]: Please create a proper table using Excel

Formatted: Indent: Left: 0.3", Line spacing: Double

Formatted: Indent: Left: 0.3"

Determination of tTotal pPolyphenolic cContent (TPC)

Total phenolic content of different extracts was assessed ~~with~~ using Folin–Ciocalteu method [6~~].~~], with ~~Phenolic concentration of extracts was estimated from a~~ gallic acid as a ~~calibration~~ reference ~~curve~~standard. To make a calibration curve, 0.5 ml aliquots of 12.5, 25, 50, 100, 200, and 400 lg/ml methanolic gallic acid solutions were mixed with 2.5 ml Folin–Ciocalteu reagent (diluted tenfold) and 2.5 ml (75 g/l) sodium carbonate. After incubation at 25^0C for 30 min, the quantitative phenolic estimation was performed at 765 nm against reagent blank by spectrophotometer. A similar procedure was adopted for the extracts as described above in the preparation of calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE)/g dry weight.

Comment [ZT16]: Provide instrument manufacturer details

Total Flavonoid

Comment [ZT17]: No capital letter

Total flavonoid was estimated using the method of [7]. A s~~S~~ample of 0.5 ml was mixed with an equal volume of 2 % AlCl3 ethanol solution which was kept for 1 h at room temperature~~.~~ . then the absorbance was measured at 420 nm. Total flavonoid content was calculated as mg quercetin/g dry weight based on calibration curve.

Comment [ZT18]: Reconstruct as new sentence and indicate instrument used and its details

Antioxidant activity determination by 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay

The DPPH scavenging assay is based on electron donation of antioxidants to neutralize DPPH radical. The reaction is accompanied with colour change of the DPPH measured at 517 nm, and the discolouration acts as an indicator of the antioxidant efficacy. The method is ~~largely~~ based on the assumption that antioxidant activity is equal to its electron~~–~~donating capacity or so-called reducing power. For measuring DPPH radical scavenging activity, 2 ml of each extract and control at various concentrations were added to 3 ml of freshly prepared DPPH solution (50 lM) in methanol [8]. The reaction was allowed for 30 min and absorbance was measured at 517 nm using a spectrophotometer. Results were expressed in mM of ascorbic acid equivalent (AAE)/g dry weight.

Comment [ZT19]: Provide apparatus details

Azino-Bis_~~(~~[3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS)] Radical Scavenging Activity ABTS Assay

Comment [ZT20]: Make sentence case

For ABTS assay, stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution [9]. The working solution was then prepared by mixing the two stock solutions in equal ~~quantity~~ quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 60 ml methanol to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using the spectrophotometer. Sample extracts (150 ml) were allowed to react with 2850 ml of the ABTS solution for 2 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. Results were expressed in mM of ascorbic acid equivalent (AAE)/g dry weight.

Comment [ZT21]: Provide instrument information

Comment [ZT22]: Provide instrument information

Ferric reducing antioxidant power (FRAP) determination Assay

The FRAP test is an ET-based approach that evaluates the reduction of ferric ion ($Fe3^{+}$)-~~–~~ligand complex to the brightly blue ferrous ($Fe2^{+}$) complex by antioxidants in acidic conditions~~ by antioxidants~~. FRAP assay was conducted using method of [10] with some modifications. 200 ml of extract were added with 3 ml of FRAP reagent that was prepared with mixture of 300 mM sodium acetate buffer at pH 3.6, 10 mM 2,4,6- tri (2-pyridyl)-s-triazine (TPTZ) solution and 20 mM $FeCl_3 6H2O$ at the ratio of 10:1:1. The reaction mixture was incubated in a water bath at 37C for 30 min. The increase in absorbance was measured using spectrophotometer at 593 nm. The antioxidant capacity, based on the ability to reduce ferric ions of the extracts, was calculated as mM GAE/g dry weight from the GAE–FRAP standard curve

Comment [ZT23]: Insert symbol for degree

Formatted: Highlight

Statistical ~~Analysis~~analysis

Data were ~~submitted~~ subjected to analysis of variance (ANOVA) and ~~significant differences in~~ mean ~~values~~ were separated using Tukey's test at α = 0.05. Statistical analyses were performed using R software [11].

Comment [ZT24]: Name the analyses one by one

Comment [ZT25]: Provide software information

Results and Discussion

Analysis of variance

Comment [ZT26]: Please present the results of ANOVA for all studied variables

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX XXXXXXXX

Total Phenolic Content and Total Flavonoids

Genotypes collected from different states of India representing different ecological zones revealed significant differences for total phenolic content (Table 2). The amount of total phenolic contents varied from 17.38 ~~(IC-561652)~~ to 131.5 mg GAE/g ~~(IC-561635) mg GAE/g~~ dry weight, recorded for IC-561652 and IC-561635, respectively~~.~~ ~~Total flavonoid content also varied significantly among the population. The maximum flavonoid content~~

~~was exhibited by IC 561731 (56.15 mg quercetin/g dry weight) however, the minimum was observed in IC 561652 (14.07 mg quercetin/g dry weight).~~ Phenolic compounds and flavonoids contribute largely for the antioxidant properties due to presence of bioactive compounds [12]. The phenolic compounds tend to inhibit lipid autoxidation by acting as radical scavengers. Phenols are compounds that have the ability to destroy radicals because they contain hydroxyl groups. These important plant components give up hydrogen atoms from their hydroxyl groups to radicals and form stable phenoxyl radicals and, consequently, are essential antioxidants that protect against the propagation of oxidative stress. Research studies indicate that hotter varieties of Capsicum contain more phenolic compounds as compared to the sweeter ones [13].

Flavonoids

Total flavonoid content also varied significantly among the population. The maximum flavonoid content was exhibited by IC-561731 (56.15 mg quercetin/g dry weight), whereas the minimum was observed for IC-561652 (14.07 mg quercetin/g dry weight). Flavonoids are also involved for their antioxidant activities because of the ability of hydrogen donation to stabilize the phenoxyl radicals formed [14] and thus play an important role as antioxidant agent and scavenge the free radical reaction. Existence of high phenol and flavonoid content in the Chilli genotypes point to their potentiality in Nutra pharmaceutical uses. The diversity in total phenolic content and total flavonoid among the genotypes could be attributed to diversity of habitat [15]

Antioxidant Activities:

Antioxidant activity varied significantly among the population which was measured through DPPH , ABTS and FRAP assay (Table 2). The diversity in antioxidant activities among the genotypes can be to their diverse chemical compositions exist in each extract. ~~Thus, the antioxidant potential cannot be prophesied only on its total phenolic content.~~ The amount of DPPH antioxidant activity varied from 0.55 mM AAE/g dry weight (IC-561652) to 5.60 mM AAE/g dry weight (IC-561635). IC-561731, CITH-HP-22 and CITH-HP-111 genotypes also recorded maximum DPPH antioxidant activity. ABTS antioxidant activity ranged between 16.03 (IC-561652) and 38.12 (IC-561635) mM AAE/g dry weight. IC-561731, LSVT Red-1 and IC-561703 also recorded high ABTS antioxidant activity. Antioxidant activity measured by FRAP assay varied from 0.80 (IC-561652) to 6.40 (IC-561731) mM GAE/dry weight. IC-561635, IC-561730 AND LSVT Red-1 are the genotypes showing maximum FRAP antioxidant activity. The results of the antioxidant capacity valuation of the chilli genotypes by FRAP, ABTS, and DPPH assays are shown in Table 2. These variances may perhaps be described by diverse analytical methods. FRAP assay measures the ability to reduce a ferric tripyridyltriazine ($Fe3^{+}$-TPTZ) to a ferrous form ($Fe2^{+}$-TPTZ) of samples [16]. ABTS and DPPH assays are based on the reduction of ~~ABTS and~~ABTS and DPPH free radicals [17] of samples. The results are in agreement with the results [18] also found variation in the results of antioxidant capacity by the DPPH assay (2.28 to 15.6 µmol TEAC g-1) and by the FRAP assay (3.99 to 84.67 µmol TEAC g-1) in chili pepper.

Comment [ZT27]: This section does not really discuss the findings of this study. Please revise and improve

Comment [ZT28]: What is more? Please indicate the quantities from previous research

Formatted: Font: Bold

Comment [ZT29]: Move to ANOVA results section

Comment [ZT30]: Delete. It does not discuss the current findings

Comment [ZT31]: You need to discuss your own findings along with findings from other researches

Comment [ZT32]: Please indicate the habitat differences for the studied genotypes in Table 1

Comment [ZT33]: Move to ANOVA results section

Comment [ZT34]: Move to ANOVA results section

Comment [ZT35]: This does not make sense. Please rephrase

Comment [ZT36]: Discuss these findings

Comment [ZT37]: Provide discussion to these findings

Comment [ZT38]: Discuss robustly your findings

Comment [ZT39]: Delete

Table-2: Mean performance of different genotypes of chilli (*Capsicum annuum* L.)

S.No	Genotypes	**Total Phenolic Content(**mg GAE/g dry weight)	**Total flavonoid** mg quercetin/g dry weight	**DPPH** mM AAE/g dry weight	**ABTS** mM AAE/g dry weight.	**FRAP** mM GAE/dry weight
1	IC-561635	131.5	54.06	5.60	38.12	4.4
2	CITH-HP-22	130.00	53.00	5.30	31.25	3.50
3	IC-561731	129.20	56.15	5.50	38.00	6.40
4	IC-561730	127.30	54.14	5.20	36.10	4.20
5	IC-572487	125.53	51.07	4.80	34.08	4.00
6	LSVT-Red-1	120.43	50.05	4.50	32.22	4.10
7	LSVT-Red-2	119.13	49.05	4.20	36.11	3.90

Formatted: Indent: Left: 0.3", Line spacing: Double

8	SK-SC-1161	118.03	48.13	4.10	35.30	3.70
9	LSVT-Red-3	118.06	47.00	4.00	34.10	3.60
10	IC-561610	114.16	45.10	3.90	33.15	3.10
11	CITH-HP-16	112.20	43.03	3.50	32.16	3.00
12	IC-561665	110.20	42.18	3.40	30.15	3.90
13	Bhut Jolokia	108.06	40.16	3.30	29.08	3.75
14	Kashmiri Long -1	107.05	40.07	3.40	29.00	3.90
15	Sel-917-111	106.37	39.05	3.90	30.11	3.60
16	IC-561639	103.21	41.00	4.10	31.22	3.00
17	IC-561661	102.26	36.16	3.30	31.13	3.40
18	IC-	100.48	29.09	4.10	20.33	2.50

Formatted: Indent: Left: 0.3", Line spacing: Double

	561622					
19	SKAU-078	97.36	28.10	4.50	31.12	3.10
20	SKAU-084	95.16	27.22	4.30	30.00	2.90
21	CITH-HP-71/13	94.20	25.98	4.00	29.09	2.70
22	Sel-680/11	92.26	25.08	3.90	32.18	2.00
23	IC-561627	82.46	29.08	5.10	32.00	2.80
24	ARCH-228	78.10	27.03	3.10	33.10	2.08
25	Guccha Mirch-1	70.30	28.21	5.10	31.21	2.00
26	IC-561614	69.14	27.05	4.80	28.15	2.60
27	CITH-HP-111	58.11	28.08	5.20	24.11	2.10
28	VOBC-	56.20	27.14	2.50	29.10	1.50

Formatted: Indent: Left: 0.3", Line spacing: Double

	0289					
29	Guchha Mirch-2	53.40	25.14	4.20	31.00	2.50
30	CITH-HP-171/13	51.30	24.11	4.10	23.10	2.70
31	SKAU-092	47.35	23.09	3.00	21.12	1.80
32	CITH-HP-17/13	45.13	25.00	3.20	22.07	2.80
33	SKAU-096	44.13	26.09	5.10	34.12	2.30
34	IC-561657	43.03	25.23	4.00	29.13	2.10
35	Jawahar Mirch	42.13	24.06	3.80	28.00	2.00
36	CITH-HP-1154-1/13	36.12	20.07	3.40	23.09	2.30

Formatted: Indent: Left: 0.3", Line spacing: Double

Formatted: Indent: Left: 0.3", Line spacing: Double

Formatted: Indent: Left: 0.3", Line spacing: Double

Formatted: Indent: Left: 0.3", Line spacing: Double

Formatted: Indent: Left: 0.3", Line spacing: Double

Formatted: Indent: Left: 0.3", Line spacing: Double

Formatted: Indent: Left: 0.3", Line spacing: Double

Formatted: Indent: Left: 0.3", Line spacing: Double

37	IC-561618	48.11	24.00	3.50	28.22	2.25
38	CITH-HP-1154	35.12	20.13	3.20	22.15	1.45
39	IC-561691	31.21	19.10	3.25	20.00	1.70
40	Sel-839-2	30.13	18.50	3.00	20.50	2.00
41	G-4	28.00	17.50	2.10	19.03	1.80
42	Goa-sel-1	23.83	17.00	2.50	18.50	1.50
43	SK-SC-1162	21.11	17.07	2.80	18.06	1.90
44	SKAU-089	20.08	16.03	2.00	17.19	1.10
45	IC-561652	17.38	14.07	0.55	16.03	0.80
	CV (%)	0.28	0.90	2.14	0.67	0.48

Distribution of genotypes into different clusters

Based upon the mean performance ~~of genotypes~~, ~~forty five~~the studied genotypes were grouped into Seven clusters (Table-3, Fig 1) ~~using R Software [11]~~. The cluster diagram and dendrogram indicated that the maximum number of genotypes fall in cluster III (11)

Formatted: Indent: Left: 0.3", Line spacing: Double

Formatted: Indent: Left: 0.3"

Comment [ZT40]: Why capitalized?

Comment [ZT41]: Is Table the diagram

Comment [ZT42]: Provide past tense word

followed by cluster I, II ~~(each 8)~~, cluster V ~~(7)~~, cluster IV ~~(6)~~, cluster VI ~~(4)~~ and cluster (1). Cluster I ~~consisted,~~involved IC-561610, CITH-HP-16, IC-561665, SKAU-111, Kashmiri Long -1, Sel-917-111, IC-561639, IC-561661. Cluster II included the following genotypes: IC-561635, CITH-HP-22, IC-561730, IC-572487, LSVT-Red-1, LSVT-Red-2, SK-SC-1161, LSVT-Red-3. Cluster III ~~included maximum genotypes~~involved CITH-HP-171/13, SKAU-092, CITH-HP-17/13, SKAU-096, IC-561657, Jawahar Mirch, CITH-HP-1154-1/13, IC-561618, CITH-HP-1154, VOBC-0289, Guchha Mirch-2. Cluster IV included the following genotypes: IC-561622, SKAU-078, SKAU-084, CITH-HP-71/13, Sel-680/~~11 ,~~11, IC-561627. Cluster V included following genotypes: IC-561691, Sel-839-2, G-4, Goa-sel-1, SK-SC-1162, SKAU-089, IC-561652. Cluster VI ~~included following genotypes~~involved ARCH-228, Guccha Mirch-1, IC-561614, CITH-HP-111 and luster VII included one genotypes IC-561731. The formation of different clusters with variable number of entries in each cluster indicated diversity among genotypes. The genotypes from different states of India were found to be scattered in different clusters, which suggested that a pattern of clustering of accessions was independent of their geographic origin.

Comment [ZT43]: Cluster what?

Comment [ZT44]: Rewrite without repeating what is already visible from the Table and Figure

Table-3: Distribution of chilli ~~(*Capsicum annuum* L.)~~ genotypes into clusters

Comment [ZT45]: Rephrase and put full stop

S. No	Cluster	No. of genotypes in the cluster	Name of genotypes
1	I	8	IC-561610, CITH-HP-16, IC-561665, SKAU-111, Kashmiri Long -1, Sel-917-111, IC-561639, IC-561661
2	II	8	IC-561635, CITH-HP-22, IC-561730, IC-572487, LSVT-Red-1, LSVT-Red-2, SK-SC-1161, LSVT-Red-3
3	III	11	CITH-HP-171/13, SKAU-092, CITH-HP-17/13, SKAU-096, IC-561657, Jawahar Mirch, CITH-HP-1154-1/13 IC-561618, CITH-HP-1154, VOBC-0289, Guchha Mirch-2
4	IV	6	IC-561622, SKAU-078, SKAU-084, CITH-HP-71/13, Sel-680/11 , IC-561627

Formatted: Indent: Left: 0.3", Space Before: 0 pt, After: 0 pt, Line spacing: single

Formatted Table

Formatted: Indent: Left: 0.3", Space Before: 0 pt, After: 0 pt, Line spacing: single

Formatted: Indent: Left: 0.3", Space Before: 0 pt, After: 0 pt, Line spacing: single

Formatted: Indent: Left: 0.3", Space Before: 0 pt, After: 0 pt, Line spacing: single

Formatted: Indent: Left: 0.3", Space Before: 0 pt, After: 0 pt, Line spacing: single

5	V	7	IC-561691, Sel-839-2, G-4, Goa-sel-1, SK-SC-1162, SKAU-089, IC-561652
6	VI	4	ARCH-228, Guccha Mirch-1, IC-561614, CITH-HP-111
7	VII	1	IC-561731

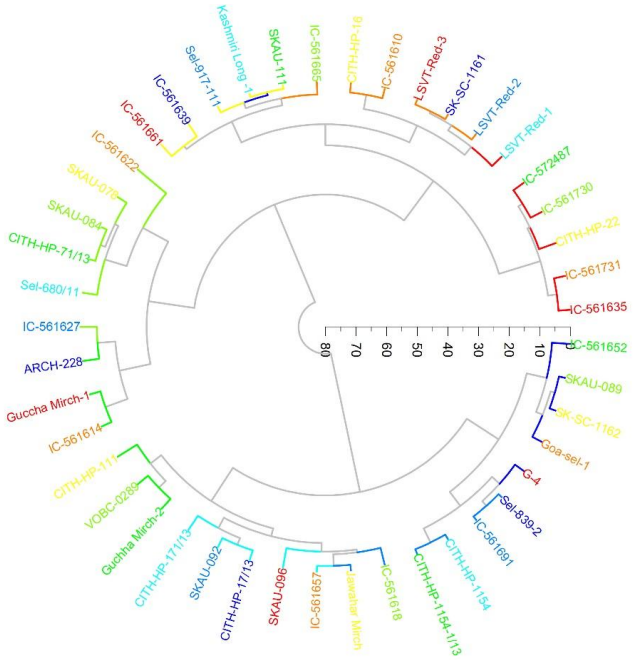

Fig 1: Dendrogram generated by hierarchical cluster analysis showing the relationships among the characterized Chilli genotypes

Identification of diverse and desirable genotypes

Non- hierarchical cluster analysis was also performed in addition to grouping of genotypes into different clusters so as to identify the diverse and desirable genotypes in terms of inter and intra cluster distances, and mean performance of clusters for various characters, respectively. ~~For this purpose, intra and inter c~~Cluster distances, ~~(Table 4)~~ and the mean performances for the studied variables ~~of each cluster for different traits was studied~~ are presented in Table 4~~.~~.

Formatted: Indent: Left: 0.3", Space Before: 0 pt, After: 0 pt, Line spacing: single

Formatted: Indent: Left: 0.3", Space Before: 0 pt, After: 0 pt, Line spacing: single

Formatted: Indent: Left: 0.3", Space Before: 0 pt, After: 0 pt, Line spacing: single

Formatted: Indent: Left: 0.3"

Comment [ZT46]: Should chilli be capitalized

The intra cluster distance ranged from 0.00 (cluster VII) to 12.30 (cluster VI) indicating ~~that~~ dissimilarity for the studied variables among the evaluated chilli genotypes ~~in clusters have dissimilarity for traits under study .~~. The members of cluster VI exhibited maximum divergence (intra cluster distance = 12.30) followed by members of cluster III (10.09). The inter cluster distance were larger than the intra cluster distances indicating a wider genetic diversity between genotypes of cluster with respect to the studied ~~traits~~ traits~~considered~~. ~~Maximum~~ A high inter-cluster distance indicates that genotypes falling in these clusters had wide diversity and can be used for hybridization programme to get better recombinants in the segregating generation. Low levels of intra-cluster distances reveal narrow genetic variation with~~-~~in cluster. Genotypes of some cluster may not provide desirable recombinants. The inter cluster distance D^2 values was highest ~~between~~ for cluster VII ~~-~~and cluster V (88.02) followed ~~by cluster~~by 81.43 for cluster V and cluster II ~~(81.43)~~.

The importance of different plant characters in the inter-cluster divergence can be studied further by comparing cluster means for different characters. Based on mean of the clusters, the donors for different characters could be selected from clusters. The cluster mean values for ~~five characters~~five characters are presented in Table-5. The perusal of data indicated considerable differences for all the characters among clusters. It is inferred from the cluster means that each cluster has its uniqueness that separated it from other cluster. Highest cluster mean for Total Phenolic Content (129.20) was ~~found~~ observed in Cluster VII while the lowest cluster mean ~~for Total Phenolic Content~~of ~~(~~24.54~~)~~ was found in cluster V. The highest cluster mean for Total ~~flavonoid (~~flavonoid (56.15) was found in cluster VII and lowest cluster mean ~~for Total flavonoid (~~of 17.04~~)~~ was ~~found~~ observed in cluster V. The highest cluster mean for DPPH (5.50) was found in cluster ~~VII~~ VII, whereas~~while~~ the lowest cluster mean ~~for DPPH (~~of (2.31) in cluster V. The highest cluster mean for ~~ABTS(~~ABTS (38.00) was found in cluster VII and the lowest cluster mean for ABTS (18.47~~)Was~~) was found in cluster V. The highest cluster mean for ~~FRAP(~~FRAP (6.40) was observed in cluster VII, whereas the ~~ile~~ lowest (1.54) was recorded in cluster IX. The h~~H~~ighest cluster mean for fruit length (13.80 cm) in cluster XII while lowest (2.60 cm) in cluster V.

Table-4: Average intra (Underlined) and inter-cluster (above diagonal) distance values in chilli (*Capsicum-annuum* L.)

Comment [ZT47]: Please revise this statement

Comment [ZT48]: First time seeing this text

Comment [ZT49]: Remove capital letters here and everywhere else applicable

Comment [ZT50]: First time reading about this trait/character/variable in this manuscript? It should show I the data collection section?

Formatted: Indent: Left: 0.3"

~~**Table-4: Average intra cluster (Underlined) and inter cluster (above diagonal) distance values in chilli (*Capsicum annuum* L.)**~~

	Group. 1	Group. 2	Group.3	Group.4	Group. 5	Group. 6	Group.7
Group. 1	7.04	17.89	48.94	16.67	66.61	31.90	24.69
Group.2		8.76	63.23	31.03	81.43	45.70	12.86
Group.3			10.09	37.97	21.17	21.18	69.68
Group.4				9.18	55.22	21.52	37.57
Group.5					9.01	38.54	88.02
Group.6						12.30	52.36
Group.7							0.00

Table-5: Cluster means for various characters in different clusters of chilli (*Capsicum annuum* L.)

		Total Phenolic	Total flavonoid	DPPH	ABTS	FRAP

Comment [ZT51]: Use "group' to avoid repeating "cluster" in the same sentence? Revise to do the same in main text.

	Content				
Cluster-I	107.94	40.85	3.60	30.75	3.46
Cluster-II	123.78	50.81	4.71	34.68	3.93
Cluster-III	45.64	24.10	3.64	26.47	2.15
Cluster-IV	93.64	27.43	4.32	29.12	2.67
Cluster-V	24.54	17.04	2.31	18.47	1.54
Cluster-VI	68.91	27.60	4.55	29.15	2.25
Cluster-VII	129.20	56.15	5.50	38.00	6.40

Relationship Among Total Phenol, Flavonoid and Antioxidant Assay

Pearson correlation analysis was performed to evaluate the suitability and reliability of the antioxidant assay for the measurement of total antioxidant activity in Chilli genotypes (Fig. 2). Correlation coefficient measures the degree of association between two or more parameters. Results revealed significant positive correlation among total phenol, flavonoid and antioxidant assay and suggest reliabilities of these methods. Significant positive correlation was found among Total Phenolic Content- Total flavonoid (r = 0.93), DPPH–ABTS (r = 0.71), ABTS–FRAP (r = 0.73) and FRAP- Total ~~flavonoid (~~flavonoid (r = 0.89). Strong positive relationship of antioxidant assays

Formatted: Indent: Left: 0.3"

Comment [ZT52]: It is not clear why correlation matrix was undertaken

Comment [ZT53]: Delete

Comment [ZT54]: Rewrite properly and include p values

suggested that all antioxidant assays used in this study are comparable and exhibit their suitability for chilli genotypes

> **Comment [ZT55]:** Add full stop

> **Comment [ZT56]:** Provide previous literature where correlations were reported in chilli or other related crops

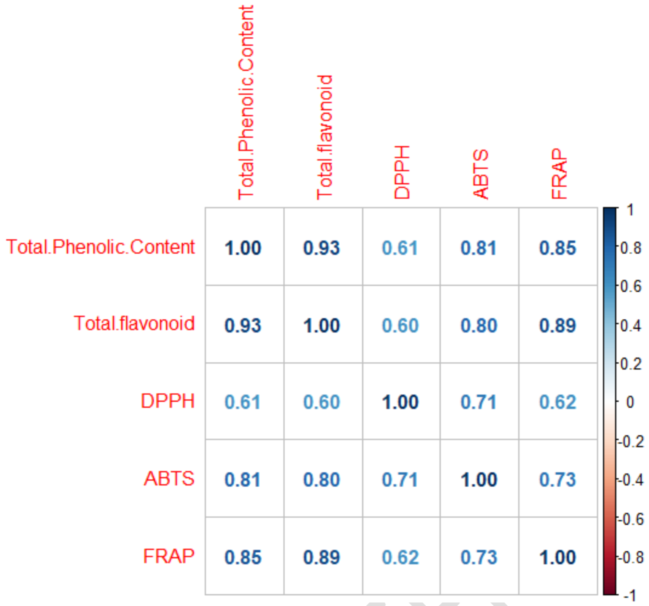

Fig. 2. Correlation coefficient between total phenol (TP), total flavonoid (TF), diphenyl-2-picrylhydrazyl (DPPH), azinobisethylbenzothiazoline-6-sulphonic acid (ABTS) and ferric reducing antioxidant power (FRAP

Principal component analysis

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

> **Comment [ZT57]:** Please include this analysis (matrix and biplots), and update the methodology, and results and discussion sections accordingly

Conclusion

The present study concludes that chilli genotypes collected from different agro-ecological zones of India displayed high content of total phenols, flavonoids and antioxidant activities. Three genotypes viz. IC-561635, CITH-HP-22 and IC-561731 exhibited highest values for all the antioxidant assays. Cluster~~ing~~ analysis revealed genotypes exhibited different ranges of antioxidants which may be used for quality breeding in Capsicum. In this context, Chilli genotype could be an important source of raw material for emerging Nutra-pharmaceutical industries and ~~brreeding~~breeding new Capsicum varieties with high antioxidant contents.

> **Comment [ZT58]:** Revise to conclude based on what the study evaluated/addressed.

Acknowledgments

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

Competing interests

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

Authors' contribution

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

References

1) WHO. COVID-19 weekly epidemiological update. World Health Organization. (2022). https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covi d-19—18-january-2022.
2) Lindgren E, Harris F, Dangour A D, Gasparatos A, Hiramatsu M, Javadi F, Loken B, Murakami T, Scheelbeek P, Haines A. Sustainable food systems—a health perspective. Sustainability Science. (2018); 13(6) :1505–1517).
3) Castro-Concha, Lizbeth A. Antioxidant capacity and total phenolic content in fruit tissues from accessions of *Capsicum chinense* Jacq. (Habanero pepper) at different stages of ripening. The Scientific World Journal. (2014) : 809073.
4) Spiller F, Alves M K, Vieria S, Carvalho T A, Leita C E, Lunardelli A. Anti-inflammatory effects of red pepper (*Capsicum baccatum*) on carrageenan and antigen-induced inflammation. J. Pharm. Pharmacol.(2008);60:473–478.
5) Alvarez-Parrilla E , De La Rosa L A , Amarowicz R , Shahidi F. Antioxidant activity of fresh and processed *Jalapeno* and *Serrano peppers*. J. Agric. Food Chem. (2011);59:163–173
6) Harborne JB. Phytochemical methods. Chapman and Hall, London. (1973) pp 49–188
7) Ordon-ez AAL, Gomez J D, Vattuone M A, Isla MI .Antioxidant activity of Sechium edule (Jacq.) Swart extracts. Food Chem. (2006); 97:452–458.
8) Susanti D, Sirat H M, Ahmad F, Ali R M, Aimi N. Antioxidant and cytotoxic flavonoids from the flowers of Melastoma malabathricum L. Food Chem. (2007);103:710–716
9) Arnao M B, Cano A, Acosta M The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. (2001); 73:239–244
10) Wong C, Li H, Cheng K, Chen F. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem. (2006); 97(4):705–711
11) R Core Team. R: A language and environment for statistical computing. (2020). R Foundation for Statistical Computing, Vienna, Austria.

Formatted: Indent: Left: 0.3"

Formatted: Indent: Left: 0.3"

Comment [ZT59]: Add these sections and others

Formatted: Indent: Left: 0.3"

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

Formatted: Highlight

12) Materska M, Perucka I. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). J Agric Food Chem. (2005): 53:1750–1756
13) Melgar-Lalanne. Oleoresins from *Capsicum spp*.: Extraction Methods and Bioactivity. Food Bioproc Tech (2017): 10(1): 51-76.
14) Sowndhararajan K, Kang S C. Free radical scavenging activity from different extracts of leaves of Bauhinia vahlii Wight & Arn. Saudi journal of biological sciences.(2013);20(4):319-25.
15) Rawat S, Jugran A, Giri L, Bhatt ID, Rawal R S. Assessment of antioxidant properties in fruits of Myrica esculenta: a popular wild edible species in Indian Himalayan region. J Evid Based Complementary Altern Med (2011):512787.
16) L. P. Leong and G. Shui. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chem. (2002);76: 69-75.
17) W. Brand-William, M. Cuelier, and M. E. Berset. Use of free radical method to evaluate antioxidant activity. Lebensm. Wiss. U. Technol. (1995);28: 25-30
18) Sora G, Haminiuk C, Silva M, Zielinski A, Goncalves G A; Bracht A, Peralta R M. A comparative study of the capsaicinoid and phenolic contents and in vitro antioxidant activities of the peppers of the genus Capsicum: an application of chemometrics. Journal of Food Science and Technology. (2015.); 52: 8086-8094

Formatted: Highlight

Comment [ZT60]: The authors need to redo this section, following journal guidelines

Formatted: Highlight

Formatted: Indent: Left: 0.3"