MOLECULAR AND PHYTOCHEMICAL CHARACTERIZATION AND OPTIMIZATION OF DORMANCY BREAKING TREATMENTS IN *CAPPARIS SPINOSA* L. FROM THE TRANS HIMALAYAN REGION OF LADAKH, INDIA.

Synopsis
of
PhD Thesis

Submitted by

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Introduction

Capparis spinosa L. (Capparidaceae) (Figure 1) also called 'Caper' and locally known as '*Kabra'* is one of the oldest known wild medicinal plant in '*Amchi system*' (local medicinal system). It has long been used by the natives of Ladakh as a leafy vegetable and forage. In India, it is found in very restricted pockets only from the inner valleys of trans–Himalaya between 3020–3890 m which includes Indus, Nubra and Suru valleys of Ladakh region. It is well adapted to the harsh climatic conditions of Ladakh, can withstand extreme temperature (–30 °C to +35 °C) and is highly drought tolerant. This plant has multiple uses in cuisine as salad, pickle and condiments. Bio-chemical studies have reported the presence of alkaloids, lipids, flavonoids and glucosinolates, cancer preventing agents and biopesticides in *Capparis spinosa*. In Ladakh, several types of medicinal preparations from *Capparis* are being used for the treatment of various ailments like gastrointestinal infection, diarrhoea and rheumatism. In Ayurveda it is used in splenic, renal and hepatic complaints; root bark is used as tonic, expectorant, anthelmintic, emmenegogue and analgesic. From ancient times, the floral buttons of *C. spinosa* (capers) were employed as flavoring in cooking and are also used in traditional medicine for their diuretic, antihypertensive, poultice and tonic properties. More specifically it is well known for its antioxidant activity and as a result it has been used in many herbal formulations. Besides pharmacological importance it is also very important for environmental safety, including soil and water conservation, desertification control and land reclamation in fragile cold ecosystem of Ladakh.

Very poor seed germination is the major problem in the wide scale cultivation of caper bush in the trans-Himalayan region because the seeds have both physical (seed coat) and physiological dormancy [1]. Fresh caper seeds germinate readily, but in low percentages (1–2%), whereas drying of seeds induces severe dormancy and is difficult to overcome naturally [2]. As the dormancy in this crop is due to the hard seed coat, external treatments are necessary to overcome the prevailing dormancy. Due to its various utility by the mankind Caper bush are being indiscriminately collected in large quantities from the Ladakh region. As a result of this and a lack of organized cultivation, there is gradual decrease in the populations of *C. spinosa* from the Ladakh region. Hence it needs immediate attention for conservation. Studies of its population structure and genetic variance are important for successful development of conservation strategies, planning any breeding experiments and commercialization of this plant. Caper buds has been reported to possess antioxidant activities [3], however, no report is available so far in the evaluation of antioxidant activities of plant parts from the Ladakh region. For the rapid development of *C. spinosa* as an economically viable alternative crop, it would be essential to evaluate the natural populations of *C. spinosa* for antioxidant activities. Furthermore, the Caper bush is used as condiments by the local tribal's, it is

utmost important to investigate the nutritional compositions, especially vitamins and micronutrients of Caper buds.

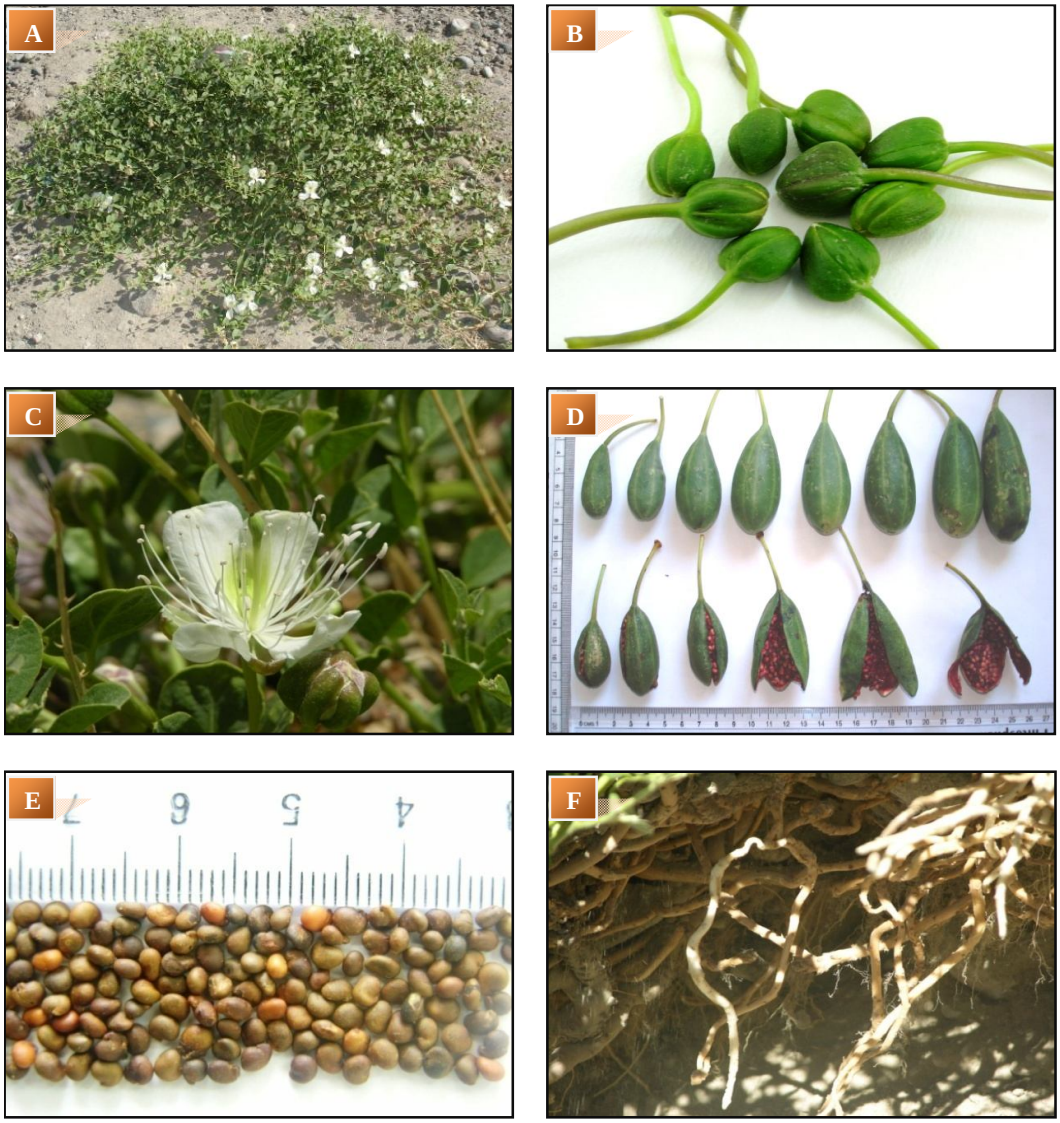

Figure 1. The plant *Capparis spinosa* and its different parts: (**A)** flowering plant at natural habitat, (**B)** flower buds, (C) flowers, (D) fruits, (E) seeds and (F) root system.

The present work is thus originated in view of the importance of *C. spinosa* to help the Indian pharmaceuticals, food and cosmetic industries to manufacture many products and drugs, to enhance the rate of seed germination, to understand genetic diversity among populations, to evaluate the antioxidant activity, to assess the nutritional content of flower buds of the populations of *C. spinosa* from the Ladakh region for commercial cultivation and realizing the existing lacunae in the study made so far in this aspect.

Objectives

- To optimize the conditions for breaking physical and physiological dormancy effectively to enhance the rate of seed germination of *C. spinosa* in Ladakh conditions for commercial propagation.

- To ascertain genetic diversity and population structure of *C. spinosa* based on DNA profiling techniques such as RAPD and ISSR in the trans-Himalayas, India for conservation strategies and future breeding program.

- To determine phytochemical compounds (phenol and flavonoid contents) and antioxidant activity of leaves collected from Ladakh region and their relationship with genetic diversity index.

- To analyze phytochemical composition (phenols and flavonoids) as well as antioxidant activity of the plant parts for pharmacological importance.

- To ascertain the nutritional composition (*viz.* minerals, vitamins and proximate content) of *C. spinosa* flower buds for formulation of nutritional supplements.

Outline of the works

Each of these pieces of work has distinct characteristics. At the same time they are related to one another. To clearly and coherently demonstrate the goal, results and conclusion of each piece of work, we have arranged each work chapter wise in a publishing format. The format will benefit readers to understand the idea of development, conclusion, coherence and full significance as each chapter will be a full manuscript from background to conclusion at publication stage.

Aim 1: Optimization of conditions for breaking seed dormancy to enhance the seed germination rate of *C. spinosa* in Ladakh conditions.

Plant materials

The seeds of *C. spinosa* were collected from the wild plants distributed in very restricted pockets in the Ladakh region. The dehisced fruits were collected in the month of October 2008. The seeds were removed from the fruits, rinsed in tap water thoroughly, dried in shade and kept at room temperature in polyethylene bags. The average seed moisture content was determined by moisture meter. The seed viability was determined by tetrazolium test using 500 seeds in five replications of 100 seed each [4].

Treatments and experimental design

The seed germination experiment was conducted as a completely randomized block design (CRBD) with four replications. Each experimental unit consists of a petri dish with 100 seeds. Seeds were surface sterilized with 0.01 % mercuric chloride, rinsed with distilled water, wash with 70 % alcohol and finally rinsed with 3 washes of MilliQ (MQ) water. The surface sterilized seeds were given various treatments as follows for the breaking of seed dormancy.

- Seeds were dipped in concentrated acids i.e. H_2SO_4, HCl and HNO_3 for 10–60 min at 10 min interval.
- Gamma rays irradiation of seeds at different doses (i.e. 10–50 KR at 10 KR interval) using the ^{60}Co gamma cell irradiator facility at the Botany Dept., RTM, Nagpur University, Nagpur, followed by dipping in concentrated H_2SO_4 for 10–60 min at 10 min interval.
- Seeds were first pretreated in concentrated H_2SO_4 for 20–60 min at 10 min interval, further dipped in GA_3 solutions (i.e. 100–500 ppm at 100 ppm interval) for a period of 1, 2, and 3 h.
- Seeds were soaked at 3 different doses of KNO_3 (i.e. 0.1, 0.2 and 0.3 %) for a period of 6, 8 and 12 h after presoaking in concentrated H_2SO_4 for 20, 30 and 40 min.
- Scarification of seeds by P320A sand paper (sand grain/cm^2) then dipped in GA_3 solutions (i.e. 200–600 ppm at 100 ppm interval) for a period of 1, 2, and 3 h.
- Seeds were stratified at -20 °C for 1–30 days at 5 day interval.
- Seeds were dipped in the hot water at 50, 60 and 70 °C for 30–120 min at 30 min interval.
- Seeds were first soaked in absolute alcohol and acetone for 1, 2, 3, 4 and 5 days and then dipped in GA_3 solutions (100–600 ppm) for a period of 1, 2, and 3 h.

Result and Discussion

Very poor seed germination is the major problem in the wide scale cultivation of capers in the trans-Himalayan region. The present study was conducted to examine the role of various dormancy breaking treatments *viz*. soaking in the warm water, scarification, stratification, Conc. acids (H_2SO_4, HNO_3, and HCl), hormone (GA_3), Potassium Nitrate, alcohol, acetone, gamma ray irradiation, etc. Highest germination rate (62%) was observed when seeds were treated with H_2SO_4 for 40 min followed by 400 ppm of GA_3 soaking for 2 hrs (Figure 2) (Table 1) and germination of 61% was achieved in case of presoaking in conc H_2SO_4 (for 20 min) followed by dipping in 0.2 % KNO_3 for 8 h (Table 2). There are possibilities that hard seed coat was not the only hurdle to the germination and the dormancy was partly due to the physiological restrictions as well. We have achieved significantly higher rate of seed germination of *Capparis spinosa* from cold arid desert of trans–Himalayan region of Ladakh. Therefore it is recommended to germinate the caper seeds in the petridishes under specified treatments and then transfer these seedlings first in the greenhouse for 2-

3 months and then to the open field conditions during spring-summer season (May-August) of Ladakh. The standardization of germination technique will ultimately help in the widespread cultivation of this wonder plant at the farmer's field of Ladakh.

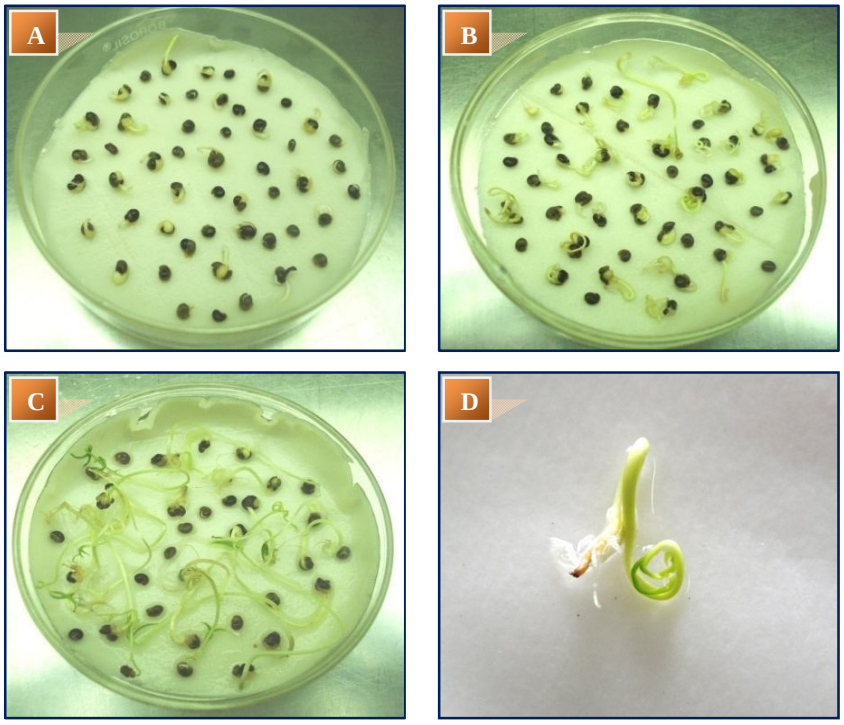

Figure 2. Effect of conc. H_2SO_4 (40 min.) and 400 ppm GA_3 (2 hr.) on caper seed germination percentage.

Table 1. Effect of concentrated H_2SO_4 presoaking (durations) along with GA_3 (concentrations and durations) on seed germination (%).

GA_3 (ppm)	Duration (h)	H_2SO_4 presoaking duration (min)					germination (%) Mean ±SEM
		20	30	40	50	60	
100	1.0	8.0	31.0	33.0	29.0	5.0	21.2±3.25
	2.0	10.0	34.0	39.0	32.0	7.0	24.4±3.54
	3.0	7.0	27.0	31.0	24.0	6.0	19.0±2.85
200	1.0	11.0	33.0	38.0	32.0	11.0	25.0±3.13
	2.0	12.0	37.0	41.0	37.0	13.0	28.0±3.46
	3.0	17.0	28.0	33.0	27.0	9.0	22.8±2.35
300	1.0	12.0	39.0	42.0	34.0	15.0	28.4±3.36
	2.0	14.0	43.0	47.0	39.0	16.0	31.8±3.78
	3.0	8.0	35.0	39.0	31.0	11.0	24.8±3.44
400	1.0	16.0	42.0	48.0	39.0	15.0	32.0±3.71
	2.0	7.0	47.0	62.0	42.0	17.0	35.0±5.42
	3.0	11.0	37.0	44.0	36.0	12.0	28.0±3.72
500	1.0	14.0	34.0	39.0	35.0	13.0	27.0±3.04
	2.0	15.0	39.0	45.0	38.0	14.0	30.2±3.53
	3.0	10.0	29.0	36.0	32.0	11.0	23.6±2.94
Mean (%)		11.47	35.67	41.13	33.80	11.67	
±SEM		±0.55	±0.88	±1.15	±0.77	±0.58	

Presoaking with H_2SO_4 ($P<0.01$); GA_3 ($P<0.01$); Duration ($P<0.01$); Presoaking with H_2SO_4 x GA_3 ($P<0.01$); Presoaking with H_2SO_4 x Duration ($P<0.01$); GA_3 x Duration ($P=0.642$); Presoaking with H_2SO_4 x GA_3 x Duration ($P<0.01$)

Table 2. Effect of presoaking treatments of concentrated H_2SO_4 (duration) in combination with KNO_3 (concentrations and durations) on seed germination (%).

H_2SO_4 presoaking duration (min)	KNO_3 concentration (%)									Mean germination (%)±SEM
	0.1			0.2			0.3			
	Soaking duration (h)			Soaking duration (h)			Soaking duration (h)			
	6	8	12	6	8	12	6	8	12	
20	38.0	12.0	12.7	27.0	61.0	13.0	34.0	44.3	13.0	28.3^{b}±3.25
30	32.0	36.0	18.0	36.0	29.0	23.0	38.0	30.0	16.0	28.7^{b}±1.51
40	8.0	11.0	13.0	21.0	16.0	11.0	21.0	9.0	12.0	13.6^{a}±0.96
Mean germination (%)	26.00^{xyz}	19.67^{wxy}	14.56^{wx}	28.00^{yz}	35.33^{z}	15.67^{wx}	31.00^{yz}	27.78^{yz}	13.67^{w}	-
±SEM	±4.62	±4.13	±1.09	±2.29	±6.70	±1.93	±2.61	±5.20	±0.94	-

$^{a, b, c}$ different superscripts in a column differ significantly ($P < 0.01$)
$^{w, x, y, z}$ different superscripts in a row differ significantly ($P < 0.01$)

Aim 2: Study of genetic variation among *Capparis spinosa* populations using RAPDs and ISSRs DNA markers

There is lack of information on the genetic diversity of caper germplasm from the Ladakh region. Most capers are gathered from natural habitat with little attention to the preservation of germplasm resources and genetic diversity. Therefore it is essential to carry out preliminary studies to assess the extent of genetic variation between and within natural population of caper. The results obtained will help in making strategies for germplasm conservation as well as molecular breeding program.

Plant materials

Fresh tender leaves of *C. spinosa* were collected from nine different locations of the Ladakh region between altitudes 3135 m to 3435 m (Table 3). All these locations are from three different valleys, *viz.* Indus, Nubra and Suru, which are geographically distinct from each other. Young leaves were frozen in liquid nitrogen and stored at -80 °C prior to DNA isolation.

Extraction and quantification of genomic DNA

Total genomic DNA was extracted from the leaf samples according to the protocol of Doyle and Doyle [5] with minor modifications. RNA was removed by RNase treatment. DNA was quantified by comparing with λ uncut DNA using 1.5% agarose gel. The DNA samples were diluted to 12.5 ng μl^{-1} and used in PCR.

Genetic characterization using RAPD markers

Twenty random decamer primers from IDT Tech, USA (Table 4) were used for RAPD amplification following the protocol of Williams *et al.* [6]. Amplification reaction were performed in volumes of 17 µl containing 1.5 µl 10 X PCR assay buffer, 200 µM of each dNTPs, 20 ng primer, 25

ng template DNA and 0.5 unit of *Taq* polymerase ('Sigma Aldrich, USA'). DNA amplification was performed using a Biometra Gradient (Germany), thermal cycler. The first cycle consisted of denaturation of template DNA at 94 °C for 5 min, primer annealing at 37 °C for 1 min, and primer extension at 72 °C for 2 min. In the next 40 cycles the period of denaturation was reduced to 1 min at 92 °C, while the primer annealing and primer extension time remained the same as in the first cycle. The last cycle consisted of only primer extension (72 °C) for 7 min. The amplification products were electrophoresed on 2.5% agarose gel containing ethidium bromide using 1X TAE buffer (pH 8.0). DNA fragments were visualized under UV light and photographed using gel documentation photographic system (Alpha Innotech, Alphaimager, USA).

Table 3. Nine populations of *C. spinosa* collected from different sites of three valleys and at different altitudes covering 90 accessions.

Name of Valley	Populations (as per collection site)	Accession No.	Altitude (meter)	Latitude (°E)	Longitude (°N)
Suru	Batalik : 1-8	1-8	3309	76.2465	34.5541
Indus	Nimmu : 9-12	9-12	3319	77.3434	34.1926
	Basgo : 13-16	13-16	3241	77.2917	34.2139
	Phyang : 17-31	17-31	3347	77.4669	34.1615
	Phey : 32-46	32-46	3185	77.4815	34.1399
	Thiksey : 47-62	47-62	3435	77.6648	34.0554
Nubra	Skampuk : 63-68	63-68	3197	77.4158	34.6383
	Skuru : 69-78	69-78	3135	77.3685	34.6485
	Tirchey : 79-90	79-90	3159	77.3513	34.6579

Table 4. List of primers used for RAPD amplification, GC content, total number of loci, the level of polymorphism and resolving power.

Primer	Nucleotide sequence (5'-3')	G+C (%)	Total number of loci	Number of polymorphic loci	Percentage of polymorphic loci	Total no. of fragments amplified	Resolving power
S-21	CAGGCCCTT C	70	07	07	100.00	426	9.46
S-22	TGCCGAGCT G	70	16	16	100.00	802	17.82
S-23	AGTCAGCCA C	60	13	12	92.30	952	21.15
S-24	AATCAG CCA C	50	14	14	100.00	992	22.04
S-25	AGGGGTCTT G	60	12	12	100.00	599	13.31
S-26	GGTCCCTGA C	70	11	10	90.90	546	12.13
S-27	GAAACGGGT G	60	14	13	92.85	703	15.62
S-28	GTGACGTAG G	60	09	09	100.00	339	7.53
S-29	GGGTAACGC C	70	10	10	100.00	486	10.8
S-30	GTGATCGCA G	60	11	11	100.00	580	12.8
S-31	CAATCGCCG T	60	06	06	100.00	323	7.17
S-32	TCGGCGATA G	60	05	05	100.00	219	4.86
S-33	CAGCAGCCA C	70	13	13	100.00	818	18.17
S-34	TCTGTGCTG G	60	11	11	100.00	634	14.08
S-35	TTCCGAACC C	60	10	10	100.00	502	11.15
S-36	AGCCAGCGA A	60	12	12	100.00	646	14.35
S-37	GACCGCTTG T	60	14	14	100.00	357	7.93
S-38	AGGTGACCG T	60	12	12	100.00	659	14.64
S-39	CAAACGTCG T	60	11	11	100.00	484	10.75
S-40	GTTGCGATC C	60	12	12	100.00	675	15.00
Total		-	**223**	**220**	**98.65**	**11742**	-

Genetic characterization using ISSR markers

The ISSR primers were obtained from 'Applied Biosciences, India' (Table 5). Amplification reaction were performed in volumes of 25 µl containing 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl, 200 µM of each dNTPs, 0.4 µM primer, 20 ng template DNA and 0.5 unit of *Taq* polymerase ('Sigma-Aldrich, USA'). Initial denaturation for 5 min. at 94 °C was followed by 40 cycles of 1 min. at 94 °C, 1 min. at specific annealing temperature (± 5 °C of Tm), 2 min. at 72 °C and a 10 min. final extension step at 72 °C. The amplification for each primer was performed twice independently in order to ensure the fidelity of RAPD and ISSR markers. Amplification products were electrophoresed on 1.5% agarose gel and documented on a gel documentation system (Alpha Innotech, Alphaimager, USA).

Table 5. List of ISSR primers used, sequence information, GC content, total number of loci, the level of polymorphism, size range of fragments and resolving power. Where, (Y = C, T; R = A, G).

Primers	Nucleotide sequence	G+C (%)	Total number of loci	Number of Polymorphic loci	Percentage of polymorphic Loci	Total no. of bands amplified	Resolving power
ISSR 1	$(AG)_8T$	47.0	16	16	100	797	17.71
ISSR 2	$(AC)_8T$	47.0	08	08	100	205	4.55
ISSR 3	$(TG)_8A$	47.0	05	05	100	56	1.24
ISSR 4	$(AG)_8YT$	47.2	10	10	100	407	9.04
ISSR 5	$(GA)_8YT$	47.2	13	13	100	509	11.31
ISSR 6	$(GT)_8YC$	52.7	16	16	100	735	16.33
ISSR 7	$(ACC)_6$	66.6	12	12	100	807	17.93
ISSR 8	$(GGC)_6$	100	05	05	100	279	6.20
Total	-	-	**85**	**85**	**100**	**3795**	-

ISSR 9-20 did not amplify with the genotypes used in the present investigation. Individual primers sequences were given in the parentheses. ISSR 9 [$(AT)_8T$]; ISSR 10 [$(TA)_8RT$]; ISSR 11[$(AT)_8YA$]; ISSR 12 [$(CT)_8$ T]; ISSR 13 [$(TC)_8$ A]; ISSR 14 [$(GT)_8$ A]; ISSR 15 [$(TGC)_6$]; ISSR 16 [$(TGCA)_4$]; ISSR 17 [$(CTAG)_8$]; ISSR 18 [$(GA)_8$ T]; ISSR 19 [$(CT)_8$ RA] and ISSR 20 [$(CCG)_8$]

Data analysis

The banding patterns obtained from RAPD and ISSR were scored as present (1) or absent (0), each of which was treated as an independent character. Jaccard's dissimilarity coefficient (J) was calculated, subjected to cluster analysis by bootstrapping and neighbor-joining (NJ) method using the program DARWIN (version 5.0.158). Genetic diversity among populations and between three major groups (with respect to valleys) was analyzed using POPGENE software (version 1.32). Data for observed number of alleles (Na), effective number of alleles (Ne), Nei's genetic diversity (H), Shannon's information index (I), number of polymorphic loci (NPL) and percentage polymorphic loci (PPL) were also analyzed. The RAPD and ISSR data were subjected to a hierarchical analysis of molecular variance (AMOVA) [7], using three hierarchical levels; among valleys, among populations and among genotypes. Statistically unbiased clustering of collected genotypes was performed using STRUCTURE (version 2.3.1).

Result and Discussion

RAPD profiling

All the chosen primers gave amplification products ranging from five (S32) to sixteen (S22), varied in size from 200–2,500 bp (Figure 3) and yielded 11742 fragments. Out of 223 amplified bands, 220 were found polymorphic (Table 4). A dendrogram analysis based on bootstrapping and neighbor joining (NJ) method grouped all the 90 genotypes separately into seven main clusters (Figure 4). The genotypes were segregated separately as per their geographical distribution, albeit small mixing among themselves. At populations level the genotypes were clustered with respect to their site of collections with bootstrap value greater than 70% (Figure 5A). To further test this population structure, a statistically unbiased clustering method (K-means clustering) was implemented in the program STRUCTURE, without prior knowledge about the populations. Under the admixed model, STRUCTURE calculated the estimate of likelihood distribution of genotypes at the population level. It was revealed that genotypes are more likely distributed (with high probability score) with respect to their populations (Figure 5B).

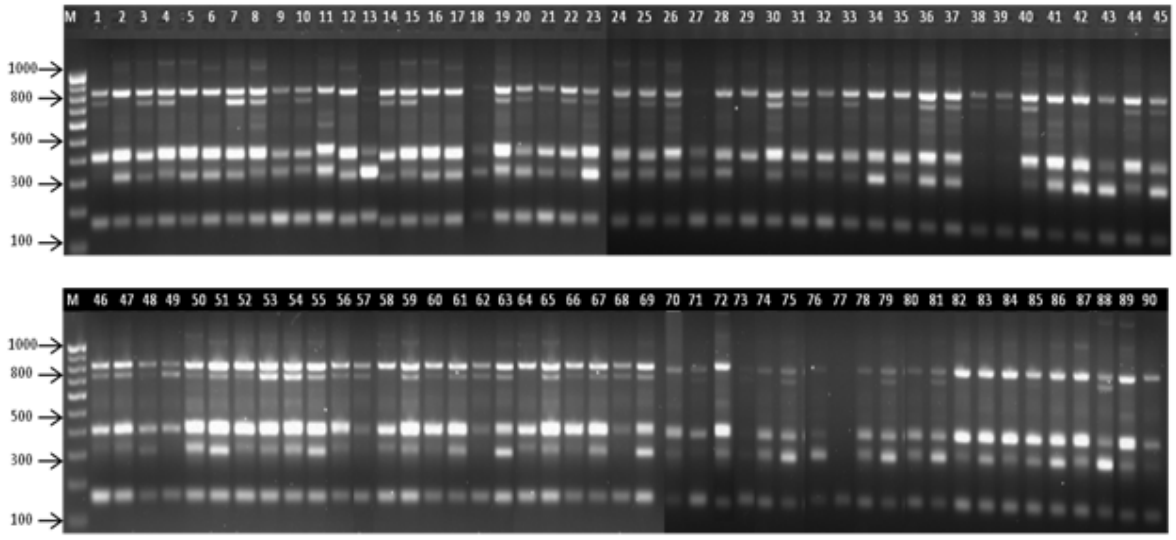

Figure 3. DNA profiling of *C. spinosa* on agarose gel, amplified with RAPD primer **S21**. 1. Batalik (1-8); 2. Nimmu (9-12); 3. Basgo (13-16); 4. Phyang (17-31); 5. Phey (32-46); 6. Thiksey (47-62); 7. Skampuk (63-68); 8. Skuru (69-78); 9. Tirchey (79-90). M = the size of molecular markers in base pairs using λ DNA.

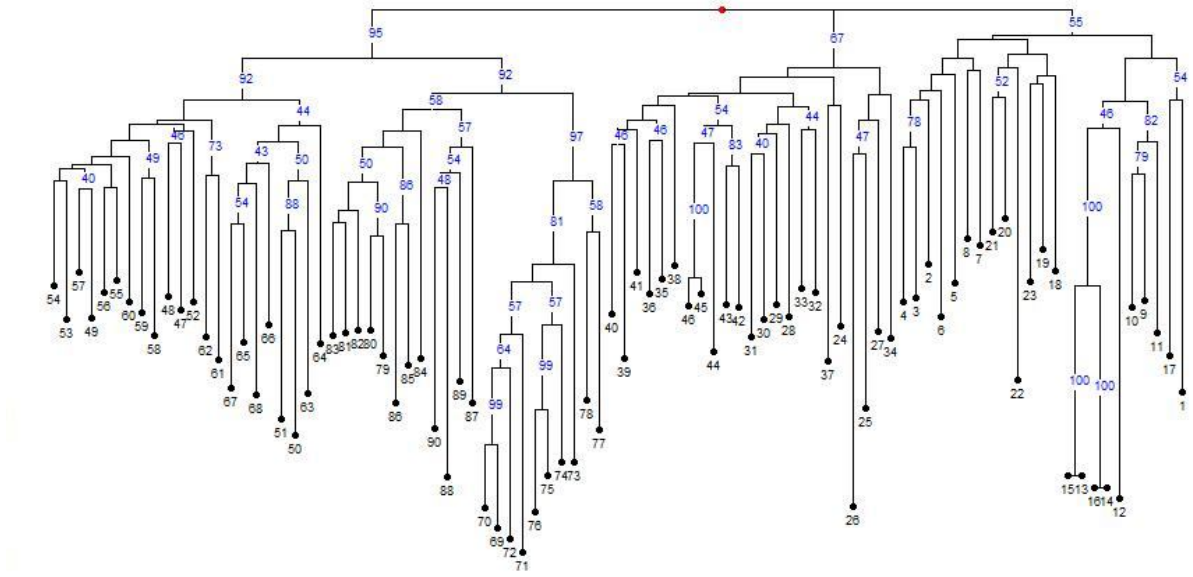

Figure 4. Dendrogram generated by Neighbor joining (NJ) clustering technique showing relationships between 90 genotypes of *C. spinosa* collected from 9 different populations, *viz.* Batalik (1-8); Nimmu (9-12); Basgo (13-16); Phyang (17-31); Phey (32-46); Thiksey (47-62); Skampuk (63-68); Skuru (69-78); Tirchey (79-90) based on RAPD profiling. Number indicates bootstrap support values.

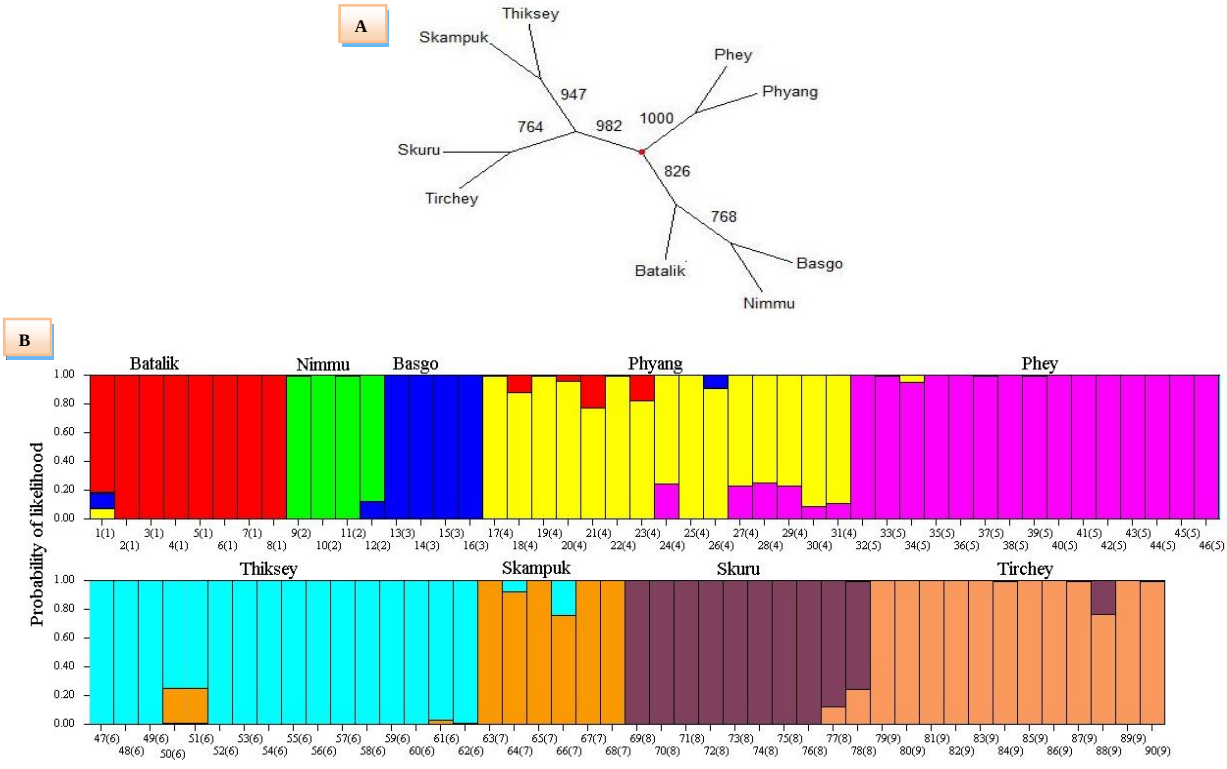

Figure 5. (A) NJ tree representing clustering of genotypes at populations' level along with supported bootstrap values based on RAPD profiling: (B) Statistically unbiased clustering of 90 genotypes at population level. The genotypes were more likely clustered with respect to their populations. The value within bracket represents the different population. Genotypes from different populations are represented with different colours: Batalik (red), Nimmu (green), Basgo (blue), Phyang (yellow), Phey (pink), Thiksey (light blue), Skampuk (brown), Skuru (deep brown) and Tirchey (light brown).

Genetic diversity analysis in terms of Na, Ne, H, I, Ht, Hs, and PPL with respect to all the three valleys revealed higher values, indicating more variability among the genotypes (Table 6). The genetic variation was high among the genotype as measured by percentage of polymorphic bands (98.65%) and Shannon information index (I= 0.56). The mean coefficient of gene differentiation (Gst = 0.411) and overall value of mean estimated number of gene flow (Nm = 0.71) revealed large gene exchanges among populations (Table 7). Analysis of molecular variance indicated that the source of variation with respect to three valleys was 5.44%, with respect to nine populations was 34.14% and with respect to 90 genotypes was 60.41%. This high level of differentiation among genotypes is probably the historical geographical and genetic isolation of populations in a harsh mountainous environment.

Table 6. Summary of genetic variation statistics for all loci of RAPD among the *C. spinosa* populations with respect to their distributions among nine sites of Ladakh region.

Name of valley	Smpling sites	Sample size	Na (mean±SD)	Ne (mean±SD)	H (mean±SD)	I (mean±SD)	Ht (mean±SD)	NPL	PPL
Suru	Batalik	8	1.659 ±0.475	1.407±0.367	0.239±0.195	0.358±0.237	0.239±0.038	147	65.92
Indus	Nimmu	4	1.457 ±0.499	1.316±0.363	0.184±0.204	0.271±0.298	0.184±0.041	102	45.74
	Basgo	4	1.157 ±0.365	1.157±0.365	0.078±0.182	0.109±0.253	0.078±0.033	35	15.7
	Phyang	15	1.865 ±0.342	1.512±0.352	0.299±0.173	0.449±0.232	0.299±0.030	193	86.55
	Phey	15	1.726 ±0.447	1.372±0.339	0.227±0.180	0.349±0.254	0.227±0.032	162	72.65
	Thiksey	16	1.686 ±0.465	1.350±0.340	0.214±0.182	0.329±0.260	0.214±0.033	153	68.61
Nubra	Skampuk	6	1.466 ±0.500	1.298±0.364	0.175±0.199	0.260±0.288	0.175±0.039	104	46.64
	Skuru	10	1.708 ±0.455	1.405±0.334	0.246±0.181	0.373±0.260	0.246±0.033	158	70.85
	Tirchey	12	1.704 ±0.457	1.376±0.348	0.228±0.183	0.349±0.259	0.228±0.033	157	70.4

Na = Observed number of alleles; Ne = Effective number of alleles; H = Nei's gene diversity; I = Shannon's Information index; Ht = Total genetic diversity; NPL = No of Polymorphic Loci; PPL = % Polymorphic Loci

Table 7. Genetic variability across all the populations of *C. spinosa*

Na	Ne	H	I	Ht	Hs	Gst	Nm	NPL	PPL	Fst	DI	EMR	MI
2.00 (00.00)	1.686 (0.281)	0.387 (0.12)	0.568 (0.143)	0.387 (0.014)	0.228 (0.008)	0.411	0.71	223	98.65	0.163	0.889	11.5	9.908

Hs = Genetic diversity in population; Gst = Genetic diversity between population; Nm= Gene flow; Fst = Wright's inbreeding coefficient; DI = Diversity index; EMR = Effective Multiplex Ratio; MI = Marker Index.

ISSR profiling

ISSR analysis yielded 85 bands, of which all are found polymorphic, with an average of 10.62 polymorphic fragments per primer, indicating a pronounced genetic variation at the genotype level (Figure 6). Also, there were high levels of polymorphism at the population level with percentage polymorphic bands (100%). The genotypes were clustered into six major clusters (I–VI) where, cluster I and III represents the genotypes from Indus and Suru valley whereas; cluster IV and

VI contains genotypes only from Indus valley. Nubra valley genotypes were clustered together in cluster II and V. However, cluster V also contains 3 genotypes from Indus valley (Figure 7). Furthermore, the genotypes were clustered into nine different populations as per their collection sites with bootstrap value greater than 75% (Figure 8A). Similarly the program STRUCTURE, without prior knowledge about the populations calculated the likelihood distribution of genotypes at the population level—genotypes are more likely distributed (with high probability score) with respect to their populations based on ISSR profiling (Figure 8B).

Genetic diversity analysis in terms of Na, Ne, H, I, Ht, Hs, and PPL with respect to all the three valleys revealed higher values, indicating more variability among the genotypes (Table 8). The genetic variation was high among the genotype as measured by percentage of polymorphic bands (100.00%) and Shannon information index (I=0.68). The mean coefficient of gene differentiation (Gst= 0.102) and overall value of mean estimated number of gene flow (Nm= 4.37) revealed large gene exchanges among populations (Table 9). Analysis of molecular variance indicated that the source of variation among three major groups (valleys) was 7.14%, among nine populations was 32.21% and among 90 genotypes was 60.63%.

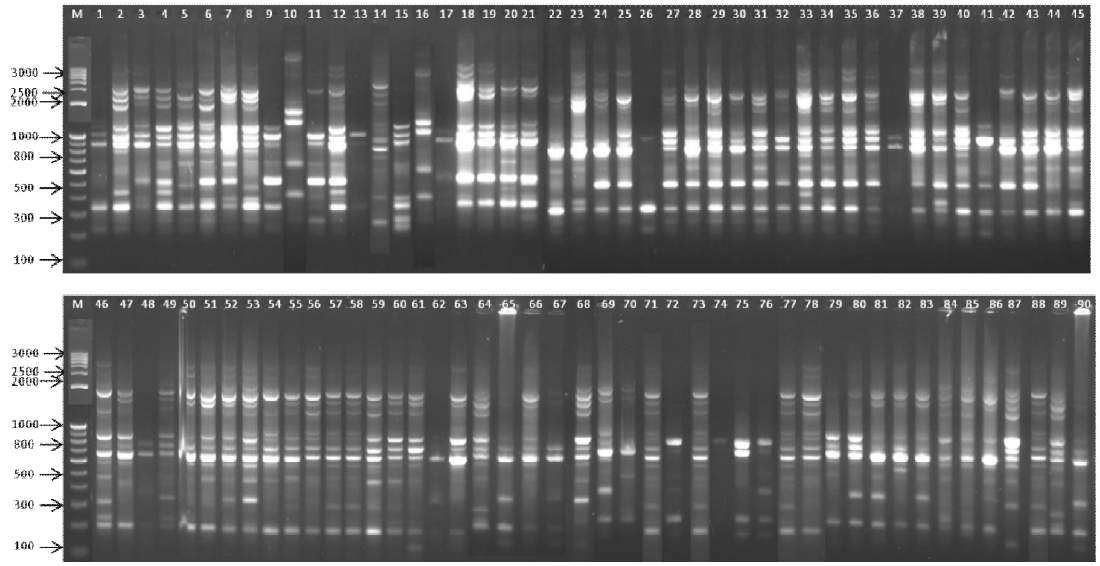

Figure 6. Profile of *C. spinosa* on agarose gel, amplified with ISSR primer S2. 1. Batalik (1-8); 2. Nimmu (9-12); 3. Basgo (13-16); 4. Phyang (17-31); 5. Phey (32-46); 6. Thiksey (47-62); 7. Skampuk (63-68); 8. Skuru (69-78); 9. Tirchey (79-90). M = the size of molecular markers in base pairs using λ DNA.

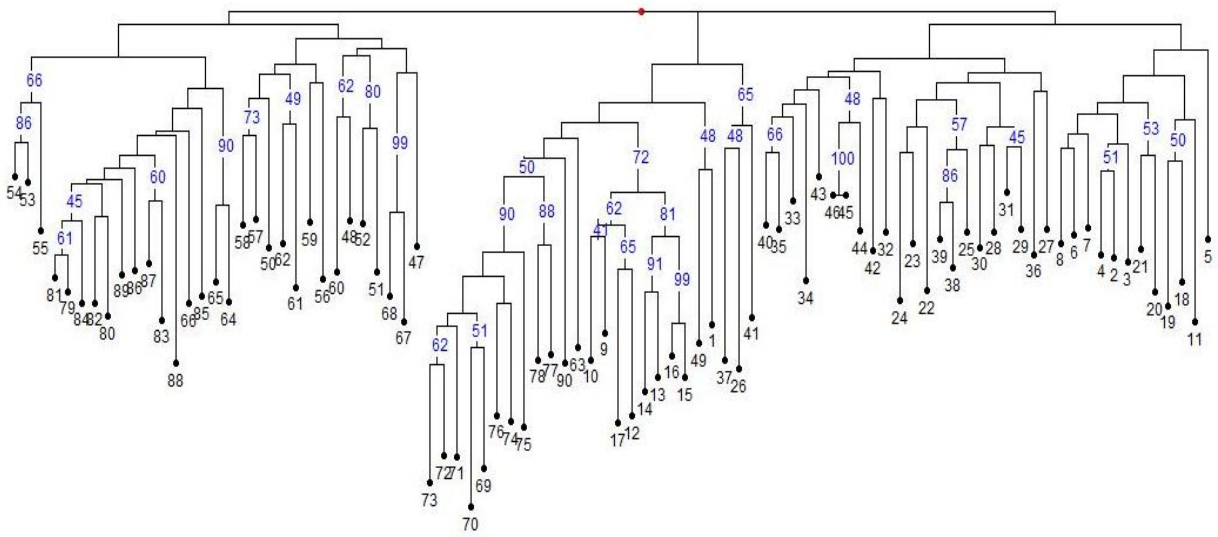

Figure 7. Dendrogram generated by Neighbor joining (NJ) clustering technique showing relationships between 90 genotypes of *C. spinosa* collected from 9 different populations *viz.* Batalik (1-8); Nimmu (N912); Basgo (13-16); Phyang (17-31); Phey (32-46); Thiksey (47-62); Skampuk (63-68); Skuru (69-78); Tirchey (79-90) based on ISSR profiling. Number indicates bootstrap support values.

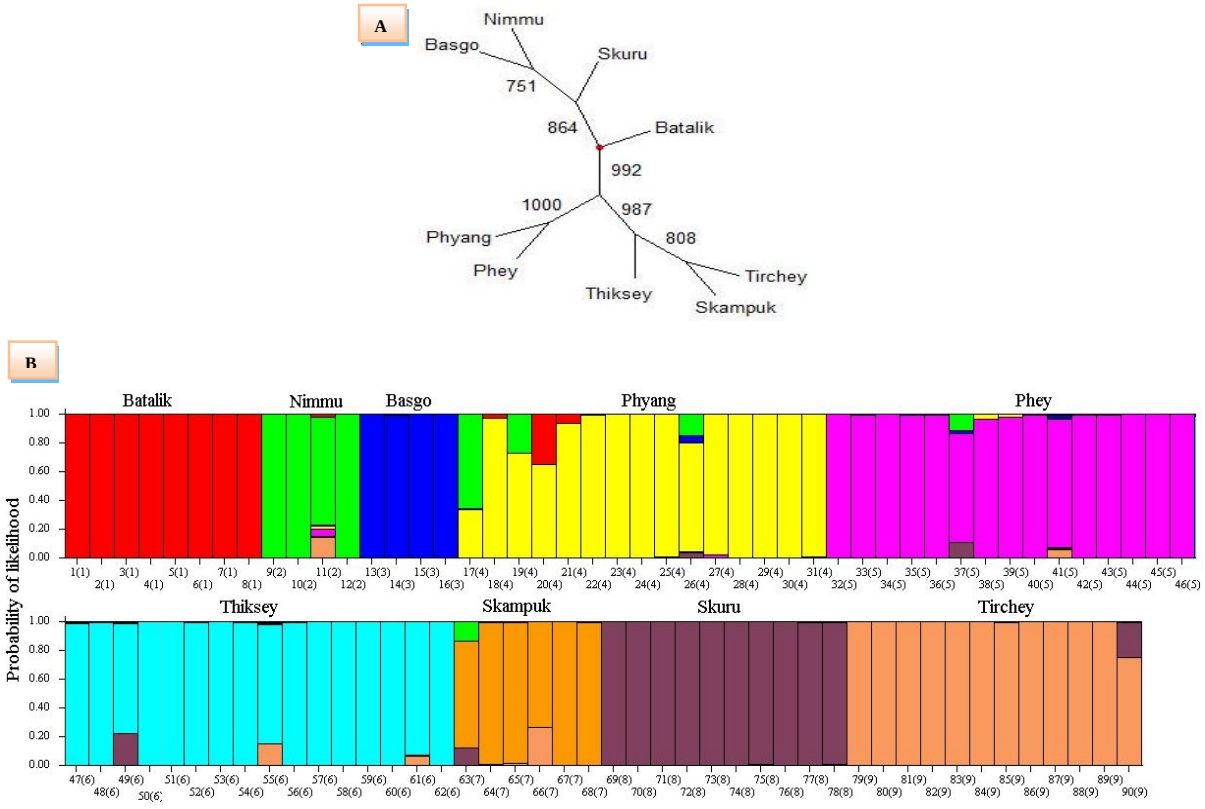

Figure 8. (A) NJ tree representing clustering of genotypes at populations' level along with supported bootstrap values based on ISSR profiling: (B) Statistically unbiased clustering of 90 genotypes at population level. The genotypes were more likely clustered with respect to their populations. The value within bracket represents the different population. Genotypes from different populations are represented with different colours: Batalik (red), Nimmu (green), Basgo (blue), Phyang (yellow), Phey (pink), Thiksey (light blue), Skampuk (brown), Skuru (deep brown) and Tirchey (light brown).

Table 8. Summary of genetic variation statistics for all loci of ISSR among the *C. spinosa* populations with respect to their distributions among nine sites of Ladakh region.

Smpling sites	Sample size	Na (mean±SD)	Ne (mean±SD)	H (mean±SD)	I (mean±SD)	Ht (mean±SD)	NPL	PPL
Batalik	8	1.963±0.190	1.772±0.248	0.420±0.110	0.603±0.143	0.420±0.012	78	96.30
Nimmu	4	1.851±0.357	1.624±0.315	0.354±0.158	0.516±0.224	0.354±0.025	69	85.19
Basgo	4	1.839±0.369	1.637±0.333	0.356±0.166	0.515±0.234	0.356±0.027	68	83.95
Phyang	15	2.000±0.000	1.853±0.167	0.455±0.058	0.646±0.063	0.455±0.003	81	100.00
Phey	15	2.000±0.000	1.892±0.124	0.469±0.038	0.661±0.040	0.469±0.001	81	100.00
Tiksey	16	2.000±0.000	1.868±0.160	0.460±0.056	0.651±0.061	0.460±0.003	81	100.00
Skampuk	6	1.963±0.190	1.765±0.251	0.418±0.110	0.600±0.143	0.418±0.012	78	96.30
Skuru	10	2.000±0.000	1.789±0.203	0.432±0.074	0.622±0.081	0.432±0.005	81	100.00
Tirchey	12	1.987±0.111	1.797±0.217	0.433±0.086	0.620±0.104	0.433±0.007	80	98.77

Table 9. Summary of genetic variability across all the 90 genotypes based on ISSR analysis.

Na	Ne	H	I	Ht	Hs	Gst	Nm	NPL	PPL	Fst	DI	EMR	MI
2.00 (0.000)	1.958 (0.054)	0.489 (0.015)	0.682 (0.015)	0.489 (0.000)	0.439 (0.000)	0.102	4.373	81	100	0.192	0.972	10.625	10.325

Genetic characterization by combination of RAPD and ISSR profiling

All the 90 genotypes collected from nine different sites (populations) were grouped into seven main clusters (Figure 9) based on bootstrapping and neighbor joining (NJ) method, whereas all the nine populations were clustered distinctly as per their geographical distribution with bootstrap value greater than 78% (Figure 10A). Based on unbiased statistical clustering technique (using STRUCTURE) the genotypes are more likely distributed as per their populations (Figure 10B). Overall the distribution of genotypes with respect to their populations was similar to that of RAPD and ISSR – most closely related populations were geographically restricted and occurred in close proximity to each other.

Genetic variation measured in terms of H, I, Ht, Hs, NPL and PPL were found higher for Nubra valley in comparison to Indus and Suru valleys (Table 10). Gst value 0.032 indicated that 96.8% of the genetic diversity resided within the population (Table 11). Hierarchical analysis of molecular variations with respect to three valleys (5.9%), among nine populations (33.61%) and among 90 genotypes (60.48%) indicated that there are more variations exist among the genotypes (Table 12) and is because of the high genome polymorphism.

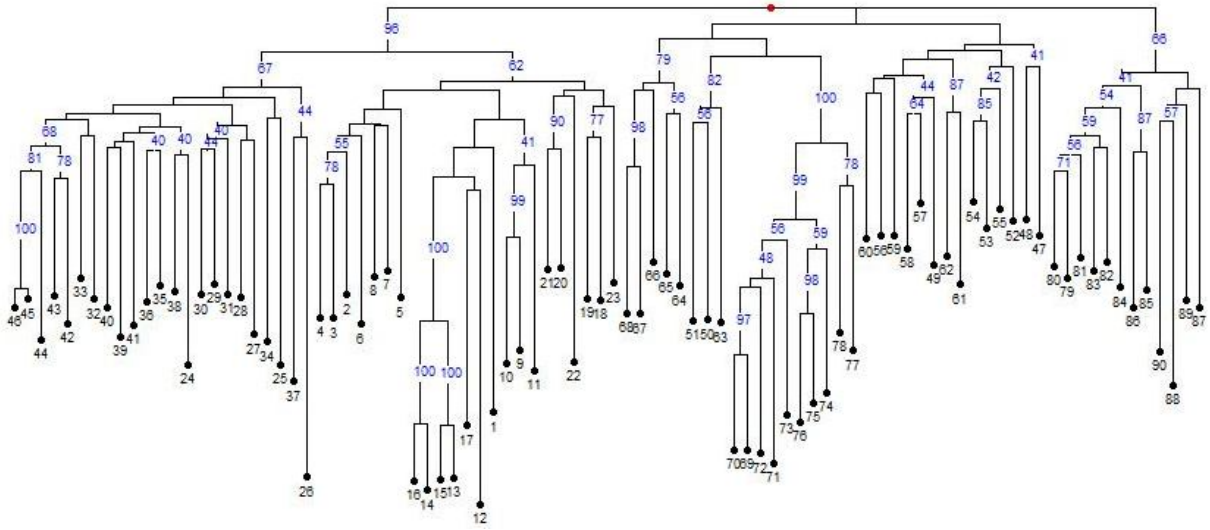

Figure 9. Dendrogram generated by Neighbor joining (NJ) clustering technique showing relationships between 90 genotypes of *C. spinosa* collected from 9 different populations *viz.* Batalik (1-8); Nimmu (9-12); Basgo (13-16); Phyang (17-31); Phey (32-46); Thiksey (47-62); Skampuk (63-68); Skuru (69-78); Tirchey (79-90) based on RAPD+ISSR profiling. Number indicates bootstrap support values.

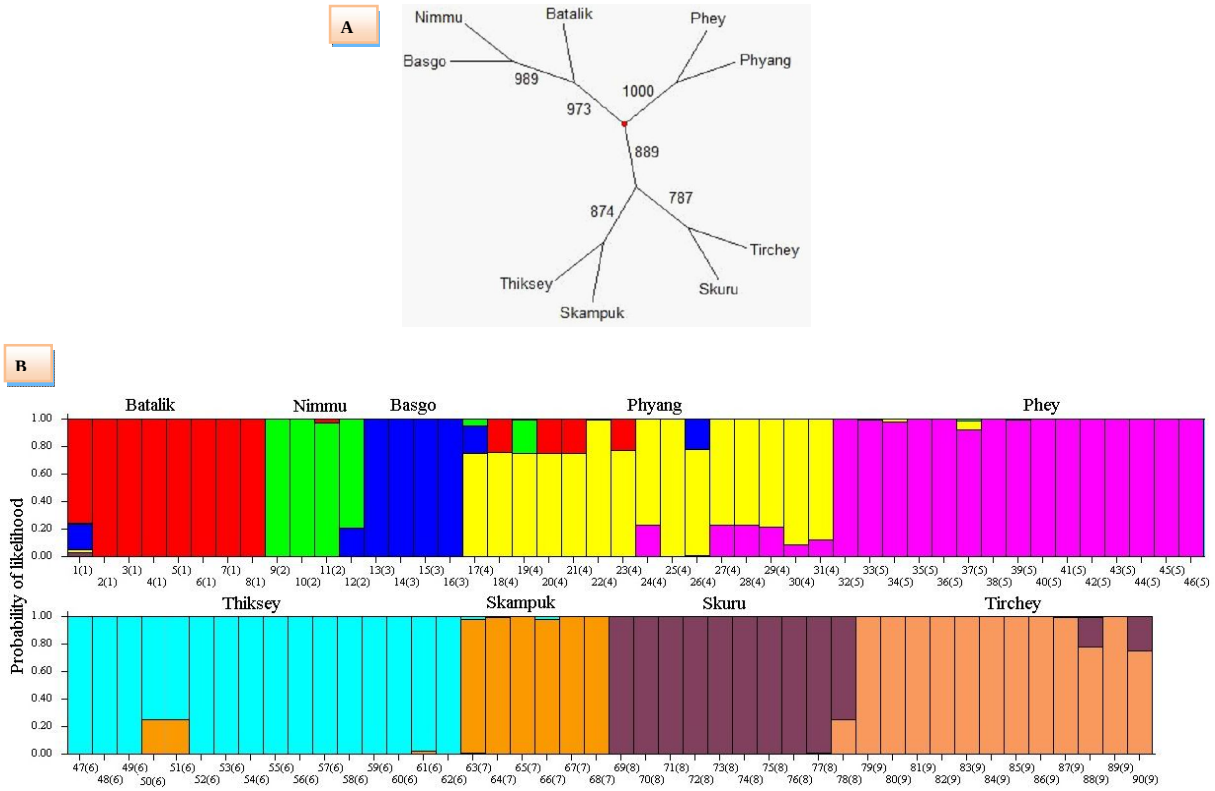

Figure 10. (A) NJ tree representing clustering of genotypes at populations' level along with supported bootstrap values based on RAPD+ISSR profiling: (B) Statistically unbiased clustering of 90 genotypes at population level. The genotypes were more likely clustered with respect to their populations. The value within bracket represents the different population. Genotypes from different populations are represented with different colours: Batalik (red), Nimmu (green), Basgo (blue), Phyang (yellow), Phey (pink), Thiksey (light blue), Skampuk (brown), Skuru (deep brown) and Tirchey (light brown).

Table 10. Summary of genetic variation statistics for all loci of RAPD, ISSR and RAPD+ISSR among the *Capparis* populations with respect to their distributions among three valleys.

Marker Type	Sample size	H (mean±SD)	I (mean±SD)	Ht (mean±SD)	PPL
RAPD					
Suru	8	0.239±0.196	0.358±0.277	0.239±0.038	65.92
Indus	54	0.35±0.142	0.52±0.18	0.350±0.02	96.86
Nubra	28	0.354±0.157	0.52±0.205	0.354±0.025	93.27
ISSR					
Suru	8	0.421±0.11	0.603±0.143	0.421±0.012	96.3
Indus	54	0.487±0.017	0.680±0.017	0.487±0.001	100
Nubra	28	0.460±0.044	0.651±0.047	0.460±0.002	100
RAPD+ISSR					
Suru	8	0.401±0.119	0.581±0.15	0.401±0.014	96.71
Indus	54	0.478±0.024	0.671±0.024	0.478±0.001	100
Nubra	28	0.483±0.022	0.676±0.023	0.483±0.001	100

Table 11. Summary of genetic variability across all the 90 genotypes based on RAPD, ISSR and RAPD+ISSR analysis.

Marker Type	H	I	Ht	Hs	Gst	NPL	PPL	Fst	DI	EMR	MI
RAPD	0.387 (0.12)	0.568 (0.143)	0.387 (0.014)	0.341 (0.012)	0.119	223	98.65	0.163	0.889	11.15	9.908
ISSR	0.489 (0.015)	0.682 (0.015)	0.489 (0.000)	0.473 (0.000)	0.033	85	100	0.192	0.972	10.625	10.325
RAPD+ISSR	0.488 (0.012)	0.681 (0.012)	0.488 (0.000)	0.473 (0.000)	0.032	308	99.02	0.136	-	-	-

Table 12. Summary of nested analysis of molecular variance (AMOVA) based on RAPD, ISSR individually and in combination, among the populations of *Capparis.* Levels of significance are based on 1000 iteration steps

Source of variation	**Among valleys**			**Among populations**			**Among genotypes**		
Markers	**RAPD**	**ISSR**	**RAPD + ISSR**	**RAPD**	**ISSR**	**RAPD + ISSR**	**RAPD**	**ISSR**	**RAPD + ISSR**
Variance component	2.450	1.211	3.661	15.377	5.459	20.745	27.227	10.275	37.503
Percentage	5.44	7.14	5.90	34.14	32.21	33.61	60.41	60.63	60.47
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

In order to determine the utility of each of the marker systems, diversity index (DI), effective multiplex ratio (EMR) and marker index (MI) were calculated according to Powell *et al*. [8]. DI for genetic markers was calculated from the sum of the squares of allele frequencies: $DI_n = 1- \Sigma\ pi^2$ (where `pi' is the allele frequency of the i^{th} allele). EMR (E) is the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay. EMR (E) = $n_p\ (n_p/n)$. MI is defined as the product of the average diversity index for polymorphic bands in any assay and the EMR for that assay, MI = DI_{avp} * E. Wright's inbreeding coefficient (Fst) was also calculated

using the programme *AFLP-SURV* [9]. Bayesian method allows the direct estimates of Fst from dominant markers without assuming previous knowledge of the degree of within-population inbreeding and that genotypes within populations are in Hardy-Weinberg proportions [10]. The marker index for ISSR (10.325) is greater than RAPD (9.908), suggesting high resolving power of ISSR in compared to RAPD. Further ISSR markers were found more efficient with regards to polymorphism detection, as they detected 100.0% as compared to 98.68% for RAPD markers. There was some consensus between the RAPD and ISSR based grouping of the 90 caper individuals.

Aim 3: Phytochemical characterization (phenol and flavonoid contents) and antioxidant activity of leaves sample collected from Ladakh region

Capparis is known to contain a wide variety of antioxidant compounds including phenolic compounds which are found to be well correlated with antioxidant potential. Phenolics have received considerable attention because of their physiological functions. The most widely used synthetic antioxidants in food (butylated hydroxytoluene: BHT) are very effective as antioxidants but their use in food products has been failing off due to suspected action as promoters of carcinogenesis. For this reason, there is a growing interest in the studies of natural healthy (non-toxic) additives as potential antioxidants. Hence, focusing our attention on natural sources of antioxidants, this study was carried out to evaluate the antioxidant activity and total polyphenolic content of *C. spinosa* tender leaves collected from nine different locations from three valleys of trans-Himalayas of Ladakh region.

Materials and methods

Extraction of total phenolic content (phenols and flavonoids)

Fresh tender leaves were collected from *Capparis* plants growing in wild from three valleys and nine villages of Ladakh during the month of July 2009 (Table 13). Leaves were air dried in shade at room temperature (26 °C) and ground to powder for extraction of total phenolic content. Ten grams of powder sample was taken and phenolic content was extracted in methanol for 72 hrs on an orbital shaker at room temperature. Extracts were filtered using a Buckner funnel and Whatman No 1 filter paper. Each filtrate was concentrated to dryness under reduced pressures at 40 ºC using a rotary evaporator. Each extract was resuspended in methanol to make 50 mg/ml stock solution.

Determination of total phenol content

Total phenol content in the extracts was determined by using modified Folin-Ciocalteau method [11]. An aliquot of the extract was mixed with 5 ml Folin-Ciocalteau reagent (previously diluted with water at 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40^{o}C for color development. Absorbance was then measured

at 765 nm using the T80+UV-VIS spectrophotometer (PG Instruments Ltd, Germany). Extracts of the samples were evaluated at a final concentration of 0.1 mg/ml. Total phenolic content was expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y=0.1216x$ ($R^2=0.9365$), where x was the absorbance and y was the Gallic acid equivalent (mg/g).

Determination of total flavonoid content

Estimation of the total flavonoids in the plant extracts was carried out using the method of Ordon Ez *et al*. [12]. 0.5 ml of sample was mixed with equal volume of 2% $AlCl_3$ ethanol solution which was kept for 1 hr at room temperature then the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extracts of the samples were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content was calculated as quercetin (mg/g) using the following equation based on the calibration curve: $y=0.0255x$ ($R^2=0.9812$), where x was the absorbance and y was the quercetin equivalent (mg/g).

Antioxidant activity of leaves extract

DPPH radical scavenging assay

Antioxidant activity of leaves extract was estimated by scavenging DPPH radical using the method of Liyana-Pathiranan and Shahidi [13]. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in methanol containing 0.02–0.1 mg of the extract. The reaction mixture left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm using BHT as reference. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = $[(Abs_{Control} - Abs_{Sample})]/(Abs_{Control})]x100$ where; $Abs_{Control}$ is the absorbance of DPPH radical+methanol; Abs_{Sample} is the absorbance of DPPH radical+sample extract /standard.

ABTS radical scavenging assay

ABTS radical scavenging assay was determined according to method of Re *et al*. [14]. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal 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 0.706 ± 0.001 units at 734 nm using spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (1 ml) were allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of BHT and percentage inhibition calculated as ABTS radical scavenging activity (%) =

[(Abs $_{Control}$ − Abs $_{Sample}$)]/(Abs $_{Control}$)]x100 where; Abs $_{Control}$ is the absorbance of ABTS radical+methanol; Abs $_{Sample}$ is the absorbance of ABTS radical+sample extract/standard.

IC_{50} (Inhibition coefficient) value was determined from the plotted graph of scavenging activity against the concentration of leaf extract collected from different sites, which is defined as the amount of antioxidant necessary to decrease the initial DPPH/ABTS radical concentration by 50%.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was conducted using method of Wong *et al*. [15]. 200 µl 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 $FeCl.6H_2O$ at the ratio of 10:1:1. The reaction mixture was incubated in a water bath at 37°C 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 percent of antioxidant. The percent of antioxidant was calculated using the formula, percent of antioxidant (%) = [(Abs $_{Sample}$ − Abs $_{Control}$)/ Abs $_{Sample}$] x100.

Table 13. Collection sites of *Capparis* leaves (along with altitude) from Ladakh region and Total phenolics as well as antioxidant activity.

Valley	Sampling sites	Altitude (m)	TPC** (mean± SD)	TFC*** (mean± SD)	ABTS IC_{50} mg/ml	DPPH IC_{50} mg/ml	FRAP* mg/ml
Suru	Batalik	3310	26.26±0.07	6.41±0.15	0.033±0.001	0.071±0.001	83.44±0.055
Indus	Basgo	3320	23.89±0.27	4.97±0.06	0.035±0.002	0.070±0.002	85.63±0.00
	Nimmu	3242	23.11±0.14	4.11±0.14	0.037±0.003	0.076±0.002	85.20±0.090
	Phyang	3347	25.15±0.00	6.20±0.13	0.032±0.003	0.076±0.002	86.77±0.070
	Phey	3185	24.78±0.73	5.69±0.07	0.039±0.003	0.072±0.002	83.38±0.055
	Thiksey	3435	23.73±0.28	4.90±0.14	0.033±0.003	0.077±0.001	85.38±0.045
Nubra	Skampuk	3197	20.12±0.14	2.90±0.20	0.037±0.003	0.073±0.001	84.42±0.050
	Skuru	3117	27.62±0.25	6.96±0.20	0.031±0.002	0.066±0.002	87.14±0.030
	Tirchey	3159	21.25±0.27	3.18±0.07	0.037±0.001	0.078±0.001	84.07±0.100

*Antioxidant contents shows at 0.1 mg/ml leaf extract.
** Expressed as mg Gallic acid/g dry plant material.
*** Expressed as mg quercetin /g dry plant material.

Result and Discussion

Total phenol and flavonoid content of leaves was extracted and analyzed in triplicate from representative plant samples of *C. spinosa* distributed into 9 geographical locations (populations). The phenols and flavonoids content in the leaves collected from Skuru were high (27.62±0.25 mg GAE/g dry wt) and (6.96±0.2 mg quercetin/g dry wt) respectively in comparison to the leaves sample collected from other locations in Ladakh region (Table 13). Whereas minimum value of total phenol (21.25±0.27) and flavonoid contents (2.90±0.2) obtained in the leaves collected from Tirchey and Skampuk respectively (Table 13). Antioxidant activity of the extract of *C. spinosa* was measured

based on different assay systems and included in Table 13. Maximum DPPH and ABTS radical scavenging activity was observed from Skuru and least from Tirchey samples. FRAP assay revealed that Skuru samples possess maximum antioxidant content as compared to the samples from any other location. The IC_{50} value of ABTS were reasonably correlated with FRAP assay (R^2=0.517). However, IC_{50} of DPPH was found poorly correlated with both ABTS (R^2=0.100) and FRAP assay (R^2=0.223). The total phenolic content and total flavonoid content were reasonably correlated with IC_{50} value of ABTS (R^2=0.741 and 0.703 respectively) and FRAP (R^2=0.605 and 0.649 respectively) but poorly correlated with DPPH IC_{50} value (R^2=0.303 and 0.408 respectively).

Relationship between variations in total phenolic content and total antioxidant activity with genetic diversity index

The variations in total phenol and total flavonoid contents of the leaves collected from 9 different populations were not significantly correlated with Nei's genetic diversity index estimated from the RAPD, ISSR and combination of both the markers (R^2=0.204 and 0.309, 0.035 and 0.079, 0.105 and 0.147 respectively). Similarly no significant relationship was obtained between the Nei's gentic diversity index and antioxidant activity measured based on ABTS assay (R^2=0.273, 0.023 and 0.070), DPPH assay (R^2=0.019, 0.024 and 0.016) or using FRAP assay (R^2= 0.035, 0.009 and 0.005).

Aim 4: Evaluation of antioxidant activities and total polyphenols from edible parts of *C. spinosa*.

There is a growing demand for natural products in the human diet, both due to the possible negative effects of synthetic food additives on human health and to the increased consumer perception. Antioxidant components are micro constituents present in the diet that can delay or inhibit lipid oxidation, by inhibiting the initiation or propagation of oxidizing chain reactions, and also involved in scavenging free radicals. A great number of edible medicinal plants contain chemical compounds that exhibit antioxidant properties. Edible parts of plant such as fruits, buds and roots are reported to contain a wide variety of antioxidant components, including phenolic compounds. However, there was no report available for the analysis of phenolic contents and antioxidant activity of edible parts of *Capparis* from Ladakh region. In the present study we investigated and compared phenolic contents and antioxidant activity from four different edible parts: leaves, buds, fruits and roots.

Materials and methods

All edible parts (such as leaves, buds, fruits and roots) were collected from *Capparis* plants in triplicate growing in wild from Ladakh during the month of July 2009. The plant was identified by its vernacular name by the local people and later authenticated at the Herbarium of Defence Institute

of High Altitude Research, Leh-Ladakh, India. The methods for extraction of polyphenols and their quantification as well as antioxidant activities were described previously.

Result and Discussion

Highest DPPH and ABTS radical scavenging activity was observed in leaves and least in dried fruit samples (Table 14). FRAP assay illustrated that leaves samples possess maximum antioxidant contents and dried fruit sample restrain minimum as compared to the other edible parts and well-known antioxidant BHT (Figure 11). The IC_{50} value of DPPH was highly correlated with IC_{50} value of ABTS (R^2=0.9084) and FRAP assay (R^2=0.9771). However, IC_{50} value of ABTS reasonably correlated with FRAP assay (R^2=0.5838). The phenolic and flavonoid content was found to be highest in leaves samples (24.78-5.69 mg GAE/g DW) and lowest in dried fruit samples (4.07-0.00 mg quercetin equivalent/g DW) (Figure 12). The total phenolic contents were highly correlated with IC_{50} value of ABTS (R^2=0.9084), DPPH (R^2=0.9388) and FRAP value (R^2=0.9618). Similarly the total flavonoid contents also were significantly correlated with ABTS (R^2=0.7449), DPPH (R^2=0.8791) and FRAP value (R^2=0.9588).

Table 14. Free radical scavenging activity (IC_{50}) value for methanolic extract of all edible parts of *C. spinosa* collected from Ladakh region (Values are expressed as mean ± SD, *n* = 3).

Edible parts	IC_{50} mg/ml	
	DPPH	ABTS
Leaves	0.050 ± 0.003^{a}	0.033 ± 0.003^{a}
F. Buds (6-8 mm)	0.068 ± 0.002^{b}	0.048 ± 0.002^{b}
F. Buds (9-12 mm)	0.091 ± 0.002^{c}	0.077 ± 0.002^{d}
Roots	0.094 ± 0.003^{cd}	0.066 ± 0.003^{c}
Fruits (Dried)	0.097 ± 0.002^{d}	0.086 ± 0.002^{e}

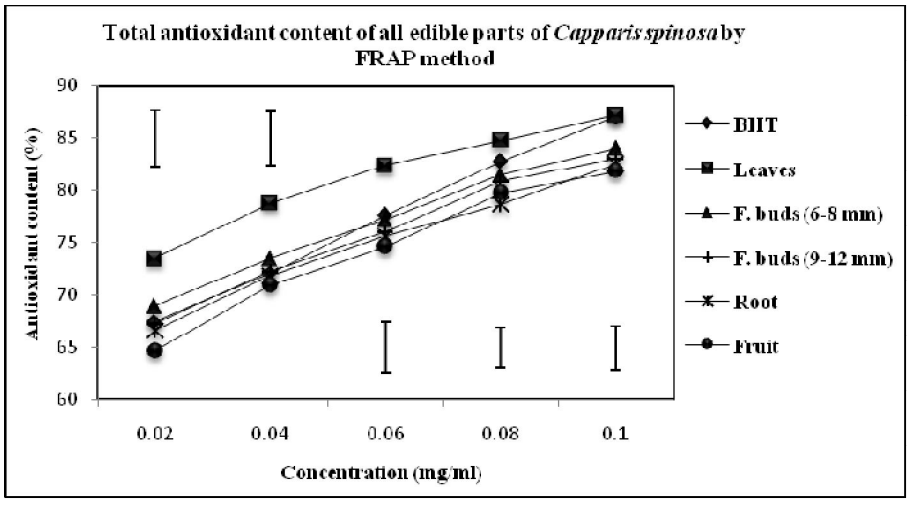

Figure 11. Antioxidant content (%) of methanolic extract from edible parts of *C. spinosa*, expressed as percent of antioxidant using FRAP method.

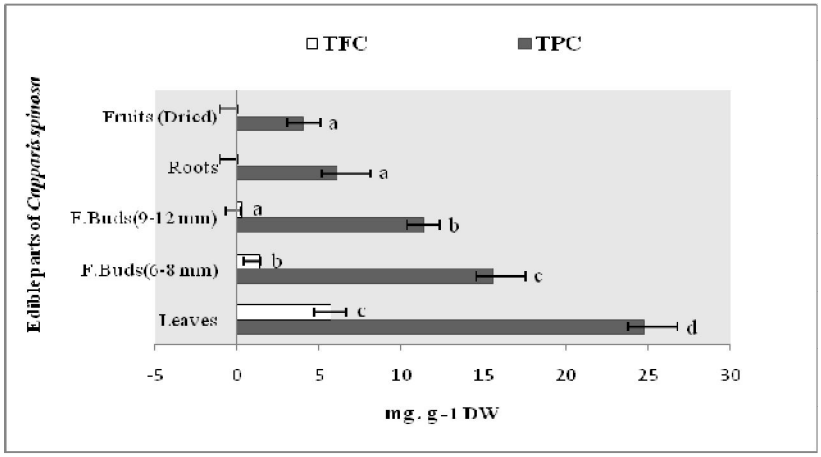

Figure 12. Total phenol content (gray bars, mg of GAE g $^{-1}$ of DW) and total flavonoid content (white bars, mg of quercetin g $^{-1}$ of DW) of *C. spinosa* edible parts.

Aim 5: Assessment of nutritional and physicochemical composition of *C. spinosa* flower buds

Nutritionally inferior diets and improper feeding practices are major contributing factors to the development of childhood malnutrition. Various reports noted that many underutilized wild edible plants are nutritionally rich and can supplement nutritional requirements, especially vitamins and micronutrients [16, 17]. The acute imbalance between the increase in the rate of population growth and the world food production has led to the present shortage of protein supply and the spread of malnutrition. Available possibilities of bridging the gap between present and future food production level and consumption are the exploitation of new nonconventional food resources and the enrichment of the poor quality foodstuffs. There is very little information published on the physicochemical composition of caper buds all over the world. Till date no report has been published on the nutritional profiling of Caper buds from the cold arid desert of Ladakh. Therefore the aim of this study was to characterize and estimate nutritional composition of *C. spinosa* buds from Ladakh region.

Materials and methods

Fresh flower buds were collected from wild plants of *Capparis* in Ladakh region in the month of July 2009, shade dried and used for proxiomate, minerals and vitamins content. The proximate (Moisture, ash, protein, carbohydrate) analyses of caper buds samples were done using the method reported by AOAC [18]. The caper buds samples were analyzed for different minerals composition using the Inductively Coupled Atomic Absorption Spectrophotometer. The organic matter in the sample was digested by taking 0.5 g of sample and 5 ml distilled concentrated nitric acid in 100 ml flask; kept overnight and next day sample were heated at 120^{o}C using hot plate till approximately 1 ml was left. Then 2 ml of 30% hydrogen peroxide were added and heated as before till

approximately 1 ml was left and diluted with distilled water. The caper buds samples were analyzed for different vitamins using the (HPLC) High Performance Liquid Chromatography. The chromatographic system was equipped with a Shimadzu HPLC and photodiode array detector. Supelcosil LC 18 DB column (250 mm×4.6 mm, 5µm; Sigma, USA) were used for separation of vitamins. The experiments were carried out in 3 replicates. Data were analyzed using complete randomized design according to Panse and Sukhatme [19].

Result and Discussion

Nutritional compositions including carbohydrates, protein, ash, moisture content, minerals and vitamins were estimated and included in Table 14. The values obtained from the Ladakh samples were also compared with the previously reported values from European countries [20]. It has been revealed that Caper buds collected from the Ladakh region were found to be a good source of protein and minerals. Thus it can meet the calorie requirement of local people and the army deployed in the 'Ladakh' sector.

Table 14. Nutritional composition (*viz.* minerals, vitamins and proximate content) of *C. spinosa* flower buds.

Proximates	**Units**	**Obtained value (per 100g)**	**Reported value * (per 100g)**
Energy	kcal	74.21	23.21
Protein	g	5.53	2.36
Ash	g	1.69	8.04
Carbohydrate	g	12.19	4.89
Fiber, total dietary	g	2.6	3.2
Total Fat	g	0.37	0.0
Moisture	g	80.22	83.85
Calcium, Ca	mg	99.18	40.00
Iron, Fe	mg	2.30	1.67
Magnesium, Mg	mg	56.32	33.00
Phosphorus, P	mg	08.58	10.00
Potassium, K	mg	4.95	40.00
Sodium, Na	mg	2.79	2.96
Zinc, Zn	mg	0.95	0.32
Copper, Cu	mg	0.23	0.37
Manganese, Mn	mg	0.42	0.07
Selenium, Se	mg	0.004	1.2
Vitamin C, total ascorbic acid	mg	5.52	4.3
Thiamin	mg	0.05	0.018
Riboflavin	mg	0.028	0.139
Niacin	mg	0.132	0.652
Vitamin B-6	mg	0.121	0.023
Folic acid	mcg	0.176	0.0
Vitamin A, IU	IU	142.3	138.0
Vitamin E (alpha-tocopherol)	mg	0.94	0.88

*** USDA Nutrient Database for Standard Reference, Release 13 (1999) NBD No. 02054**

Conclusions

- Seed germination was increased significantly by different types of treatments suggesting effective breaking of physical and physiological dormancy. The described method will ultimately help in the widespread cultivation of this wonder plant at the farmers' field of Ladakh.

- High genetic variation was observed in the genotypes of *Capparis*, which implied the need to conserve sufficiently large populations in natural habitats for conservation of its genetic diversity and avoidance of genetic erosion.

- The leaves extract of *Capparis* plant contain significant amount of polyphenols and antioxidant activity. The variation in polyphenols and antioxidant activity among different populations is not significantly related with genetic variations measured based on RAPD, ISSR and in combination of RAPD and ISSR markers.

- All the edible parts including leaves of *C. spinosa* also contain significant amount of polyphenolic compounds and possess a strong antioxidant/free radical scavenging activity. The leaves and caper buds contain high amount of polyphenols and antioxidant activity.

- Flower buds of *C. spinosa* grow in Ladakh region having more nutritional contents than the other parts of the world. Therefore it can be used as nutritional supplements.

Abbreviations:

ABTS:	2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid
BHT:	butylated hydroxytoluene
CRBD:	Completely Randomized Block Design
DNA:	Deoxyribose Nucleic Acid
DPPH:	2,2-Diphenyl-2-picrylhydrazyl
DW:	Distilled Water
FRAP:	Ferric Reducing Antioxidant Power assays
GA_3:	Gibbrelic Acid
GAE:	Gallic Acid Equivalent
H_2SO_4:	Sulfuric Acid
HCl:	Hydrochloric Acid
HNO_3:	Nitric Acid
IC_{50}:	Inhibition coefficient
ISSR:	Inter Simple Sequence Repeats
ISTA:	International Seed Testing Association
KNO_3:	Potassium Nitrate
KR:	Kilo Rads
MQ:	Milli Q
NJ:	Neighbor Joining
RAPD:	Random Amplified Polymorphic DNA
RNA:	Ribose Nucleic Acid
USDA:	United State department of Agriculture

Journal Publications

(1) Mishra G.P., Singh R., **Bhoyar M.S**., Singh S.B., "*Capparis spinosa: Unconventional potential food source in cold arid desert of Ladakh*". Current Science, 96, 1563-64, 2009.

(2) **Bhoyar M.S**., Singh R., Mishra G.P., Stobdan T., "*Lesser-known plants to enrich vegetable basket in Ladakh*". Indian Horticulture, 54 (4), 16-17, 2009.

(3) **Bhoyar M.**S., Mishra G.P., Singh R., Singh S.B., "*Effect of various dormancy breaking treatments on the germination of wild caper (Capparis spinosa L.) seeds from the cold arid desert of trans-Himalayas*", Indian Journal of Agricultural Science. 80 (7), 620-624, 2010.

(4) **Bhoyar M.S**., Mishra G.P., Naik P.K., Srivastava R.B., "*Estimation of antioxidant activity and total phenolics among natural populations of Capparis spinosa leaves collected from cold arid desert of trans-Himalayas*", Australian Journal of Crop Sciences, 5(7), 912-919, 2011.

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(7) **Bhoyar M.S**., Mishra G.P., Naik P.K., Murkute A.A., Srivastava R.B., "*Genetic variability studies among three natural populations of Capparis spinosa from cold arid desert of trans-Himalayas using DNA markers*", 2011. (Biological Research) *(In Press)*

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(9) **Bhoyar M.S**., Mishra G.P., Naik P.K., Srivastava R.B., "*Estimation of antioxidant activity and total phenolics among natural populations of Capparis spinosa edible parts from cold arid desert of trans-Himalayas*", 2011. (Journal of Food Sciences) *(under review)*

Conference Publications

(1) **Bhoyar M.S.,** Mishra G.P., Naik P.K., Murkute A.A., Singh S.B., "*Assessment of Genetic construction of Capparis spinosa L. using RAPD markers*". Book of Abstracts, 4th Indian Horticulture Congress 2010 (18-21 Nov. 2010), organized by The Horticuture Society of India, N. Delhi. p. 39. 2010.

(2) **Bhoyar M.S.,** Mishra G.P., Naik P.K., Srivastava R.B., "*Genetic diversity among populations of Capparis spinosa as detected by ISSR analysis and its relationship with antioxidant activity*". Book of Abstracts, International conference on Emerging trends on food and health security in cold desert (23-25 Sept., 2011), Organized by DIHAR, DRDO, Leh- Ladakh, Jammu & Kashmir, India. p. 59, 2011.

(3) **Bhoyar M.S.,** Mishra G.P., Naik P.K., Srivastava R.B., "*Estimation of antioxidant activities and total polyphenols from various edible parts of Capparis spinosa L. from trans-Himalayas*". Book of Abstracts, International conference on Emerging trends on food and health security in cold desert (23-25 Sept., 2011), Organized by DIHAR, DRDO, Leh-Ladakh, Jammu & Kashmir, India. p. 76, 2011.

(4) Mishra G.P., Singh R., **Bhoyar M.S.,** Singh S.B., "*Response of various Pre-sowing treatments on the germination of Capparis spinosa seeds trans-Himalayas*". Souvenir, National Conference on Seabuckthorn and Environment: High Altitudes Perspective (Sept 25-27, 2009), Organized by DIHAR, DRDO, Leh-Ladakh, Jammu & Kashmir, India. p. 24, 2009.

Award/Fellowship/Honors:

(1) Award of **'DRDO Fellowship'** (2007).

(2) **'Best Presentation'** award in **Science Club Activities** on 29th April, 2010 at DIHAR Conference Hall.

(3) **'Best Poster Award'** for the poster entitled as "*Genetic diversity among populations of Capparis spinosa as detected by ISSR analysis and its relationship with antioxidant activity***"** presented during International conference on "Emerging trends on food and health security in cold desert" **(**23-25 Sept. 2011**)** Organized by DIHAR, DRDO, Leh- Ladakh, Jammu & Kashmir, India.

***Capparis spinosa* sequence submitted in NCBI.**

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