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MH Khan
Advanced Research Station for Saffron and Seed Spices, SKUAST-Kashmir, Pampore, Jammu and Kashmir, India

NA Dar
Advanced Research Station for Saffron and Seed Spices, SKUAST-Kashmir, Pampore, Jammu and Kashmir, India

BA Alie
Advanced Research Station for Saffron and Seed Spices, SKUAST-Kashmir, Pampore, Jammu and Kashmir, India

GH Mir
Advanced Research Station for Saffron and Seed Spices, SKUAST-Kashmir, Pampore, Jammu and Kashmir, India

SA Dar
Dryland Agricultural Research Station, SKUAST-Kashmir, Budgam, Jammu and Kashmir, India

AA Lone
Dryland Agricultural Research Station, SKUAST-Kashmir, Budgam, Jammu and Kashmir, India

Uzma Fayaz
Advanced Research Station for Saffron and Seed Spices, SKUAST-Kashmir, Pampore, Jammu and Kashmir, India

MT Ali
Advanced Research Station for Saffron and Seed Spices, SKUAST-Kashmir, Pampore, Jammu and Kashmir, India

S Gulzar
Advanced Research Station for Saffron and Seed Spices, SKUAST-Kashmir, Pampore, Jammu and Kashmir, India

A Khan
Advanced Research Station for Saffron and Seed Spices, SKUAST-Kashmir, Pampore, Jammu and Kashmir, India

AH Bhat
Advanced Research Station for Saffron and Seed Spices, SKUAST-Kashmir, Pampore, Jammu and Kashmir, India

Corresponding Author:
MH Khan
Advanced Research Station for Saffron and Seed Spices, SKUAST-Kashmir, Pampore, Jammu and Kashmir, India

Chemical Composition and antioxidant activity of essential oils from Kala zeera (*Bunium persicum* Bioss.) grown in temperate ecologies of J&K

MH Khan, NA Dar, BA Alie, GH Mir, SA Dar, AA Lone, Uzma Fayaz, MT Ali, S Gulzar, A Khan and AH Bhat

Abstract
Bunium persicum (Boiss) is economically an important medicinal crop growing wild in the dry temperature regions in Iran. Several isolated molecules of this species can be used as antifungals as well as in pharmaceutical industry. This investigation was performed to evaluate the degree of variability of essential oil constituents of *Bunium persicum* accessions originated from three districts Kishtwar, Bandipora, Pulwama. Among the studied populations the main compounds of essential oil in *B. persicum* were observed to be cumin aldehyde (40-41%) and γ-Terpinene (26–28%). The Pearson correlation coefficient between the essential oil contents of different studied samples indicated that p-Cymene had a highly significant positive correlation with Cumic aldehyde. The accessions were classified into three main categories according to essential oil composition. The populations showed little variation in antioxidant property ranging from 79.14% (Pulwama population) to 87.70% (Bandipora population) while the Kishtwar population exhibited 81.99% activity with overall average of 82.94%. The findings of this research can be used for sketching efficient breeding programs for this species.

Keywords: GC-MS, antioxidant activity, essential oil, principal component *Bunium persicum*

Introduction
Free radicals are the highly reactive molecules that lead to the oxidation of different biomolecules (protein, amino acids, lipid and DNA) that ultimately results in cell injury and death (McCord, 2000) [20]. These oxidative reactions reduce the shelf life of processed and fresh food stuffs and thus a serious issue in food industry (Sokmen *et al*., 2004) [33]. Thus, Antioxidants are regarded as substances which when incorporated in food products mostly lipids and lipid-containing foods can enhance shelf life of foods. The underlying mechanism in increasing the shelf life of food products is due to retardation of lipid peroxidation that is the major cause of food products deterioration during processing and storage (Singh and Marimuthu, 2006) [30]. Different synthetic products such as butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), and propyl gallate (PG) have been employed for the purpose of food preservation from the beginning of the twentieth century. However, restrictions are being imposed on the usage of these compounds because of various deteriorative effects of carcinogenicity (Madhav and Salunkhe, 1995) [19]. These effects can be completely removed by using natural antioxidants present in foods. Consequently, there arose a need to isolate, identify safe and natural sources of food antioxidant that provides an alternative to synthetic antioxidants (Wanasundara and Shahidi, 1998) [34]. Thus, the studies on identification of natural antioxidants, especially of plant origin, has enormously increased in recent years (Goli *et al*., 2005; Yasoubi *et al*., 2007; Chohan *et al*., 2008; Lopez *et al*., 2007) [14, 36, 8, 17]. *Bunium persicum* (Boiss) Fedtesch, commonly known as black cumin or Kala zeera belonging to Apiaceae family is economically an important medicinal crop growing wild in the dry temperature regions in Iran. "It is mostly used as a carminative and spice for flavoring purposes in food industry (Pourmortazavi *et al*. 2005; Gincarlo *et al*. 2006)" [13]. The seeds of *B. persicum* possess expectorant, stimulant, antispasmodic and diuretic properties and reported to have medicinal importance as commonly used for curing numerous diseases as dyspepsia, diarrhea, fever and stomach problems (Sardari *et al*. 1998; Chauhan 1999; Kala 2003; Panda 2004; Boskabady and Moghaddas 2004) [28, 6, 16, 23, 3].

Essential oils obtained from seeds of Kala zeera are considered as natural food additives and have been proved to possess multifunctional antioxidative, antibacterial, antifungal, and anti-inflammatory activities (Angioni *et al*., 2004; Singh *et al*. 2004) [1, 31]. However, various factors, including climatic, geographic, and growth stage of harvested plants, can have a significant impact on essential oil yield, content, and biological qualities (López *et al*., 2007) [17]. Thus, research on essential oil chemical variability in response to environmental and geographical conditions may provide insight into what causes chemical polymorphism (Ruberto and Baratta, 2000; Sokmen *et al*., 2005) [27, 32] Furthermore, deciphering the chemical composition of essential oils is a critical quality requirement for their marketing and contributes to their value. The aims of this work were: (i) to determine the chemical composition by using GC–MS, (ii) to evaluate the antioxidant activity by using the 2, 2′-diphenyl 1-picrylhydrazyl (DPPH)

Material and Methods

Plant material

The plant material was obtained from three districts Kishtwar, Bandipora, Pulwama. Oils were extracted by hydro distillation using Clevenger-type apparatus for 3 h (Du *et al*., 2009) [9]. The oils were dried over anhydrous sodium sulphate and kept at −4 °C until it was used.

GC-MS analysis

GC-MS analysis of each sample was carried out on Agilent 7890 AGC, furnished with an HP-5 MS capillary column (30 m × 0.250 mm, 0.25 Mm) and an HP 5975 C mass selective detector was employed for the analysis. Helium was used as the carrier gas with flow rate of 1.00 ml/min. Column temperature was initially programmed at 50 0C held for 3 minutes then increased to 150 0C at the rate of 3 0C/minute and finally increased to 250 0C at the rate of 10 0C/min. Sample was diluted in hexane 1:100 v/v of which 1.0 µl was injected automatically in split less mode. Injected with a constant temperature of 260 0C through an autosampler injector. The ionization energy was 70 eV and mass range of 40–500 AMU. The management of the GC-MS system, parameter settings for GC and mass spectrometry, and data receipt and processing were performed using Shimadzu Realtime Analysis. The compounds were identified by using NIST library.

Antioxidant activity

Antioxidant activity of different Kala zeera populations were estimated through DPPH free radical scavenging assay according to the method of Chanda and Dave (2009). 1.5 ml of freshly prepared 0.04% W/V DPPH in 80% methanol was added to 1mL of aqueous extract of each Kala zeera sample and the solution was allowed to react in dark for 30 min at room temperature. Control solution (methanol + 1.5 ml DPPH) was prepared for comparison and the absorbance was read spectrophotometrically at 517 nm. Tests were performed in triplicates and the scavenging activity of extract was expressed as the percentage of DPPH radicals scavenged.

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

Statistical analysis

Cluster analysis, Principal Components Analysis (PCA) was performed using R-software Cophenetic correlation coefficient was calculated to evaluate capability of the cluster analysis. PCA using correlation matrix was carried out to further analyze the structure of the genetic diversity and first and second principal components (PC1 and PC2) were bipolted for the ecotypes. Factor analysis using principal component method was also performed by SAS software, version 9.0 (SAS Institute Inc., Cary, North Carolina, USA). Cluster analysis and PCA were performed with the software of NTSYS-pc computer program

Results and Discussion

Yellow and dark yellow essential oils of the dried seeds were obtained from eight populations of *B. persicum*. The chemical constituents of these essential oils were investigated by GC/MS. The essential oils analyses, allowed the identification of 9 volatile constituents, accounting for 95.5–99.0% of the total oil composition (Table 1-5). Among the studied populations the main compounds of essential oil in *B. persicum* were observed to be cumin aldehyde (40-41%) γ-Terpinene (26–28%), p-cymene (15-16%), 1,4-p-Menthadien-7-al (10-11%) and D- limonene (1.71-1.80%), respectively, whereas, 5-(2-Iodoanilino)-6-(1-pyrrolidinyl) and Cyclooctasiloxane, hexadecamethyl were reported as the minor compounds of *B. persicum* essential oil that collected from different regions (Fig. 1). The present results are in accordance with the previous studies on chemical composition of *B. persicum* that were in conformity with the results of the present study. All oils contained cumin aldehyde, p-cymene and γ-terpinene as a main compound. *Foroumadi et al*. (2002) [12] identified the chemical composition of the essential oil obtained from the fruits by using GC and GC/MS. Among the 25 components identified in this oil the major constituents were reported to be cuminaldehyde (27.0%), ϒ-terpene (25.8%), P-cymene (12.14%), cuminyl alchohol (6.0%) and limonene (5.1%). Mazidi *et al*., (2012) [37] analysed essential oils using gas chromatography-mass spectrometry showed that ϒ-terpene (28.16-31.13% w/w), cuminaldehyde (24.85-29.20%), p-cymene (14.67-16.50%) and limonene (16.13-8.28%) were their main constituents with a similar composition both after HD and MAHD extraction.

In order to investigate the chemical compositions of essential oils of *B. persicum* fruit into perspective, a hierarchical cluster analysis was carried out (Fig. 2). The cluster analysis revealed at least three different group: A, the populations-1 with composition of essential oil ranking: Bicyclo[3.1.0]hexane,4-methylene-1-(1-methylethyl) > 3-p-Menthen-7-al > p-Cymene >Cumic aldehyde> D-Limonene; B, γ-terpinene > cuminic alcohol > cumin aldehyde > p-cymene > limonene > safranal; C, the population-2: Cumic aldehyde >1,4-p-Menthadien-7-al > Cyclooctasiloxane, hexadecamethyl > 3-p-Menthen-7-al. While as in population 3 beta.-Myrcene> D-Limonene > gamma.-Terpinene > 1,4-p-Menthadien-7-al. However, therapeutic effects of B. persicum ascribe to three compounds of γ-terpinene, cuminic alcohol and cumin aldehyde (Fig. 2).

The Pearson correlation coefficient between the essential oil contents of different studied samples indicated that p-Cymene had a highly significant positive correlation with Cumic

aldehyde. Also, Cumic aldehyde, 1,4-p-Menthadien-7-al and D-Limonene possesss positive correlation with p-Cymene. While as, significant negative correlation was observed between 1,4-p-Menthadien-7-al with Cyclooctasiloxane, hexadecamethyl (Fig. 3).

Data obtained from GC analyses were subjected to unsupervised pattern recognition chemometric analysis utilizing PCA to improve the visualization of these differences. The results of the PCA, as represented by the obtained score plot effectively discriminated the populations into three clusters along the first component (PC1), the second component (PC2) and that account for 99.95%, 0.04% and 0.001% (Table-5; Table-6). From the obtained results, it is obvious that populations from Kishtwar and Bnadipora are gathered together in one cluster in the right left quadrant are very closely related to each other. However, PC1 successfully discriminated between Cumic aldehyde, gamma-Terpinene with negative values of PC1 to the rest essential oils (Fig. 4).

Our results were supported by the finding of previous workers that mentioned essential oil content of herbs were significantly affected by environmental factors or growing locations (Yanive and Palevitch, 1982; Omidbaigi and Bastan 2007; Omidbaigi and Arvin, 2009) [35, 22, 21]. Several authors have been studied the effective factors on the composition of secondary metabolites in different plant species. They reported important agents that influenced on chemical composition of essential oils such as plant genetics (Shafie, 2009) [29] climate, elevation and, in general, by the environmental conditions (Purohit and Vyas, 2004; Ložienė and Venskutonis; 2005) [26, 18]. Especially in aromatic plants the essential oil profiles are remarkably affected by numerous habitat factors (Flamini *et al*., 2004) [11]. Furthermore, a close correlation between chemical groups and mountain/seaside habitat was also found, which suggested that essential oil profile composition could be dependent to altitude and exposition (Mazidi, *et al*.., 2012) [37].

Antioxidant activity of phytochemicals

Antioxidant activity of food is an essential feature for preventing oxidative damage and inhibiting lipid oxidation (Pisoschi *et al*., 2021) [24]. A useful tool for estimating a compound's potential antioxidant activity is its ability to reduce other substances. DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging activity is an efficient and simple method for determining the antioxidant ability of food samples. It accepts electrons or hydrogen atoms from antioxidants and can convert them into more stable molecules. In the present study, antioxidant activity of the Kala zeera populations were measured using the DPPH method. The populations showed little variation in antioxidant property ranging from 79.14% (Pulwama population) to 87.70% (Bandipora population) while the Kishtwar population exhibited 81.99% activity with overall average of 82.94% (Table 7 and Fig. 5). This minor variations in the antioxidant activity amongst different populations of Kala zeera might be due to the fact that same population have been distributed to various temperate ecologies of Jammu and Kashmir with the passage of time or it may be due to phylogenetic constraints or niche conservativeness (Hodkinson *et al*., 1998) [15]. Little variation in antioxidant properties and radical scavenging abilities of Kala zeera populations observed in current study may also be due to the reasons that chemical composition and bioactive components like the available phytoconstituents also showed non-significant variations. Earlier findings on antioxidant activities in Kala zeera revealed that the populations with high percentages of aldehydes and terpene hydrocarbons show highest antioxidant capacity (Chizzola *et al*., 2014, Fayaz *et al*., 2022) [7, 10]. Similar findings were also observed in the present study, populations with high gamma terpinene, D-limonene and p-cymene contents showed highest antioxidant activities (Bandipora population).

In this research, we observed differences in the amount of essential oil content, main and minor compounds between studied *B. persicum* populations According to our study and after comparison with previous researches on the composition of *B. persicum* fruit oil, it can be stated that big differences existed that indicating the possible occurrence of chemotypes in this species. Differences may be ascribed both genetically variation (Existence of chemotypes) and environmentally determined fluctuation (soil, climate). Geographical situation and weather conditions of various growing locations that studied in this experiment were different, and these results confirmed the reports based on the environmental factors affected on the quantity and quality of active substances of essential oil bearing. It is interesting to note that there is significant diversity of chemical compositions of the essential oil for wild populations of *B. persicum* in different geographic regions and altitudes. The differences in the oil content and composition of the populations could be attributed to their genetic variability and they could be a good genetic source for breeding purposes.

Table 1: Location details of Kala zeera populations

S No	District	Collection site	Altitude (mts)	Coordinates (degree)
1	Kishtwar	Padder valley	1640	33.15 N, 76.09 E
2	Bandipora	Gurez valley	2580	34.63 N, 74.83 E
3	Pulwama	Ladhoo	1592	34.02 N, 74.93 E

Table 2: Essential oil composition in Kala zeera (Kishtwar population)

Compound	Retention Time	Area %	Similarity	Base m/z
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	7.831	0.38	83	93.00
p-Cymene	8.772	15.06	95	119.10
.gamma.-Terpinene	9.383	26.83	97	93.05
L-Fenchone	9.925	0.29	83	81.10
3-p-Menthen-7-al	11.659	0.60	83	79.00
Cumic aldehyde	12.378	40.14	96	133.05
4-Isopropylcyclohexa-1,3-dienecarbaldehyde	13.070	0.74	88	79.00
1,4-p-Menthadien-7-al	13.128	10.34	94	79.05

(4R,5S)-4-Hydroxymethyl-5-hydroxytricyclo[4.4.0.0(3,8)]d	13.230	0.82	54	80.00
2-tert-Butyltoluene	13.290	0.25	57	133.15
5-(2-Iodoanilino)-6-(1-pyrrolidinyl)furazano[3,4-b]pyrazine	21.170	0.09	39	281.15
D-Limonene	15.736	1.69	87	73.05
Cyclooctasiloxane, hexadecamethyl-	18.201	0.18	87	355.00
Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	27.320	0.21	46	207.00
Cyclohexasiloxane, dodecamethyl-	13.449	0.33	86	341.00
Cyclodecasiloxane, eicosamethyl-	23.347	0.45	73	73.00
Cyclononasiloxane, octadecamethyl-	21.133	0.78	79	73.05
.beta.-Myrcene	28.017	0.43	62	73.00
2,6-Dihydroxybenzoic acid, 3TMS derivative	29.695	0.06	49	73.00

Table 3: Essential oil composition in Kala zeera (Bandipora population)

Compound	Retention Time	Area%	Similarity	Base m/z
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	7.825	0.26	95	93.05
.beta.-Myrcene	8.129	0.83	95	93.10
1,4-p-Menthadien-7-al	8.764	10.19	96	119.10
Cyclohexene, 1-methyl-5-(1-methylethenyl)-	8.855	0.43	87	93.10
Eucalyptol	8.905	0.18	85	81.05
Cumic aldehyde	9.378	40.71	97	93.05
3-p-Menthen-7-al	11.638	0.55	95	109.10
p-Cymene	12.355	15.59	96	133.05
D-Limonene	13.032	1.78	94	79.05
.gamma.-Terpinene	13.100	27.49	96	79.05
Caryophyllene	14.978	0.38	95	91.05
Cycloheptasiloxane, tetradecamethyl-	15.732	0.30	87	281.05
Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-	16.220	0.31	94	69.05
1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-m	17.095	0.47	88	91.05
Cyclooctasiloxane, hexadecamethyl-	18.198	0.08	87	73.05
9,11-Octadecadienoic acid, methyl ester, (E,E)-	24.739	0.07	85	67.00
6-Octadecenoic acid, methyl ester, (Z)-	24.810	0.11	88	55.05
1-Bromo-3,7-dimethyl-2,6-octadiene	26.903	0.28	80	69.05

Table 4: Essential oil composition in Kala zeera (Pulwama population)

Compound	Retention Time	Area%	Similarity	Base m/z
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	7.826	0.31	95	93.05
.beta.-Myrcene	8.131	0.18	95	93.05
p-Cymene	8.764	15.71	96	119.10
D-Limonene	8.848	1.73	96	68.05
Eucalyptol	8.900	0.16	72	81.10
.gamma.-Terpinene	9.377	26.53	97	93.05
Fenchol	10.348	0.29	94	81.05
(3E,5E)-2,6-Dimethylocta-3,5,7-trien-2-ol	11.178	0.13	86	67.05
3-p-Menthen-7-al	11.640	0.45	94	109.10
1,4-p-Menthadien-7-al	12.356	11.57	96	133.05
4-Isopropylcyclohexa-1,3-dienecarbaldehyde	13.035	0.67	94	79.05
Cumic aldehyde	13.100	41.77	96	79.05
Caryophyllene	14.980	0.18	93	79.05
Cyclooctasiloxane, hexadecamethyl-	25.124	0.35	71	355.05
(1S,5S)-4-Methylene-1-((R)-6-methylhept-5-en-2-yl)bicyclo	16.222	0.12	90	69.05
1-Bromo-3,7-dimethyl-2,6-octadiene	26.912	0.19	79	69.05

Table 5: Principal component analysis (PCA) scores between 9 different compound and 3 temperate micro-climates of J&K

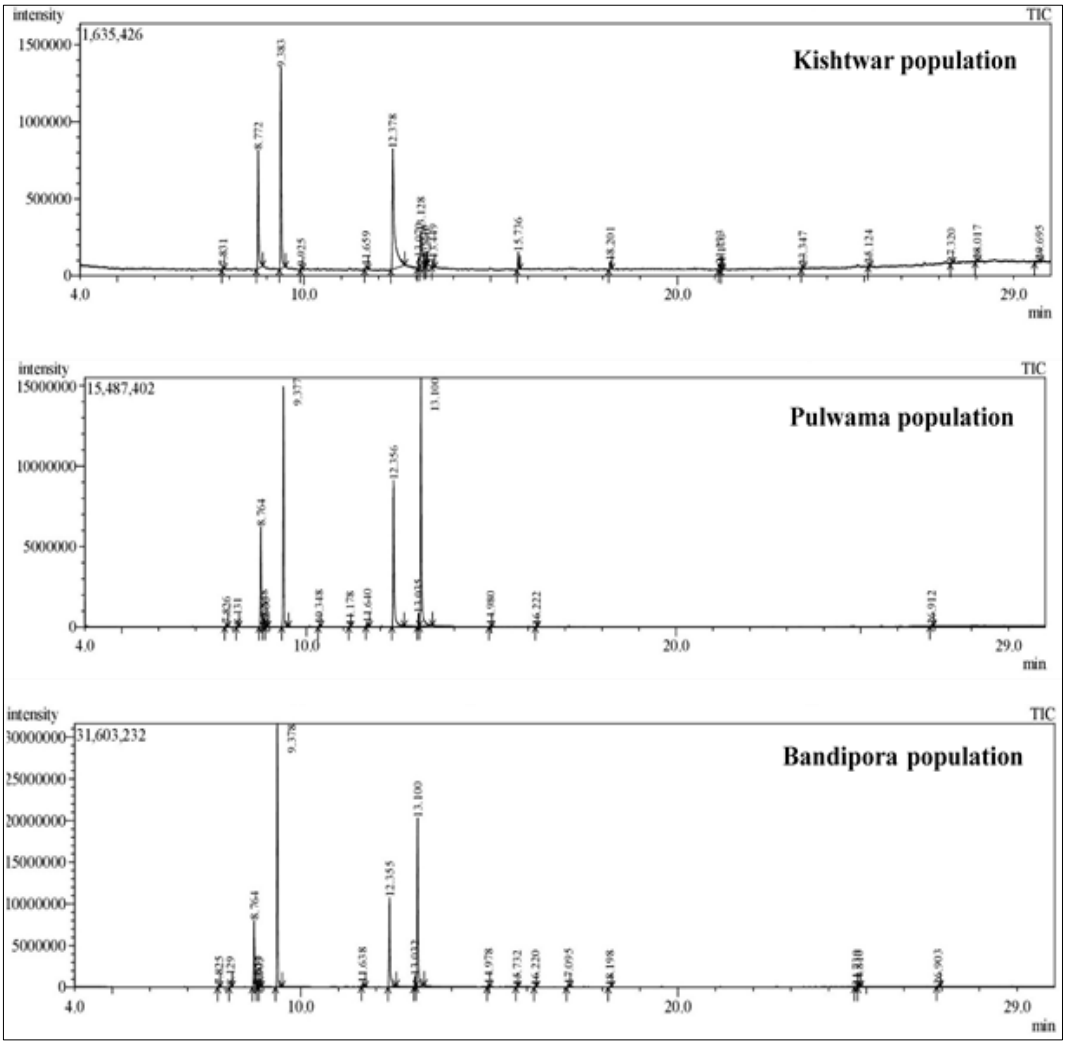
Compound	PC 1	PC 2	PC 3
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	-1.2447	-0.0008	0.0073
p-Cymene	0.5516	-0.0005	-0.0127
.gamma.-Terpinene	1.9167	0.0665	0.0070
3-p-Menthen-7-al	-1.2189	0.0064	0.0058
Cumic aldehyde	3.5683	-0.0279	-0.0003
1,4-p-Menthadien-7-al	-0.0129	-0.0623	0.0022
D-Limonene	-1.0766	0.0026	-0.0012
Cyclooctasiloxane, hexadecamethyl-	-1.2583	-0.0131	0.0032
.beta.-Myrcene	-1.2252	0.0289	-0.0113

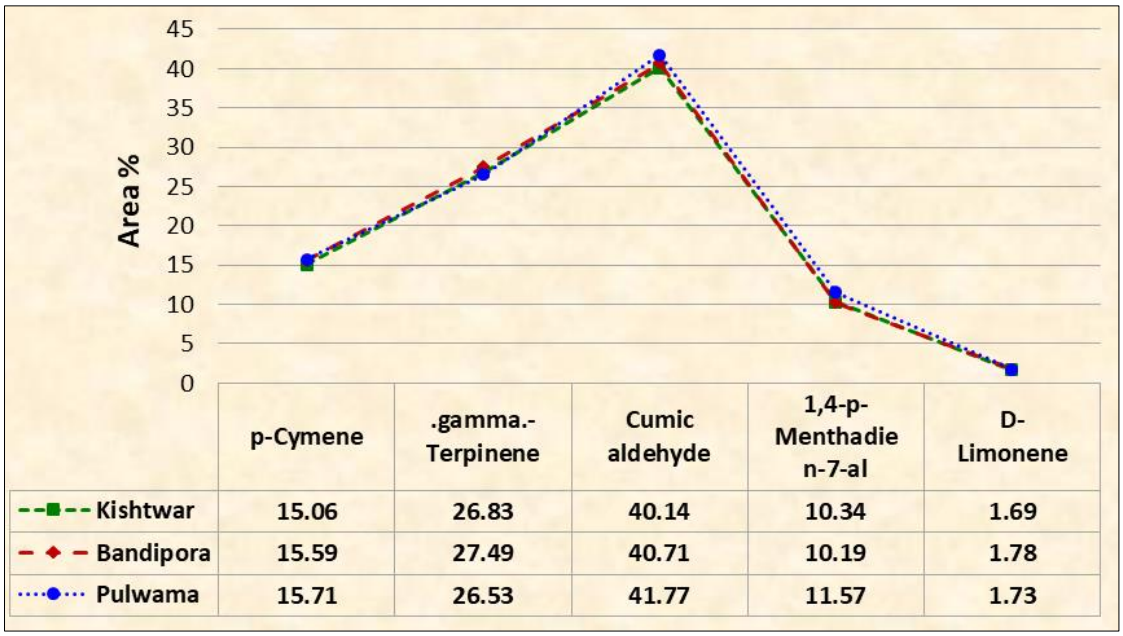
Table 6: Eigen value and percent (%) variance of three principal component (PC) that includes 3 temperate micro-climates of J&K

PC	Eigen value	% Variance
Kishtwar	2.99868	99.95592
Bandipora	0.00127	0.04224
Pulwama	0.00006	0.00184

Table 7: Antioxidant activity of Kala zeera populations

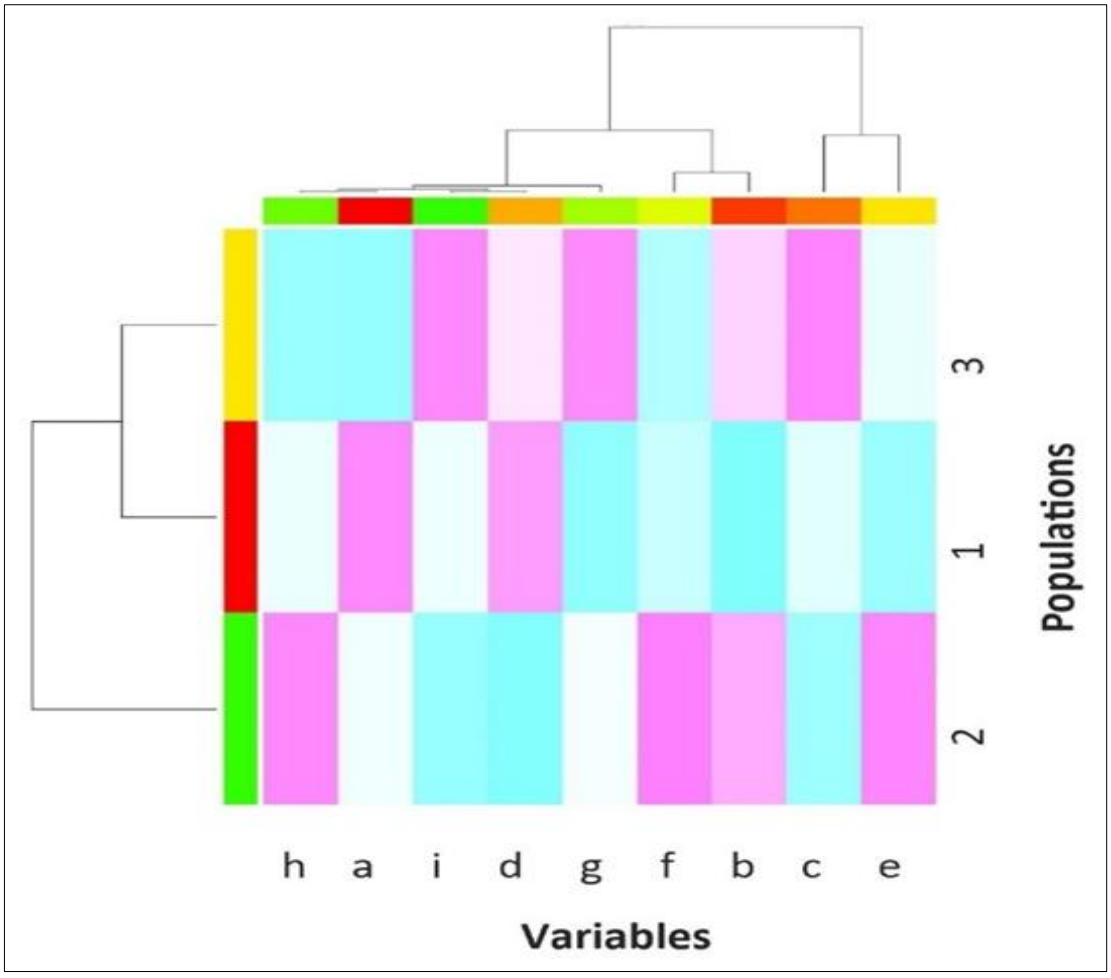
S No	Populations	Absorbance	% Radical scavenging activity
1	Kishtwar	0.069	81.99
2	Bandipora	0.101	87.70
3	Pulwama	0.117	79.14
4	Control	0.561	-
Mean			82.94





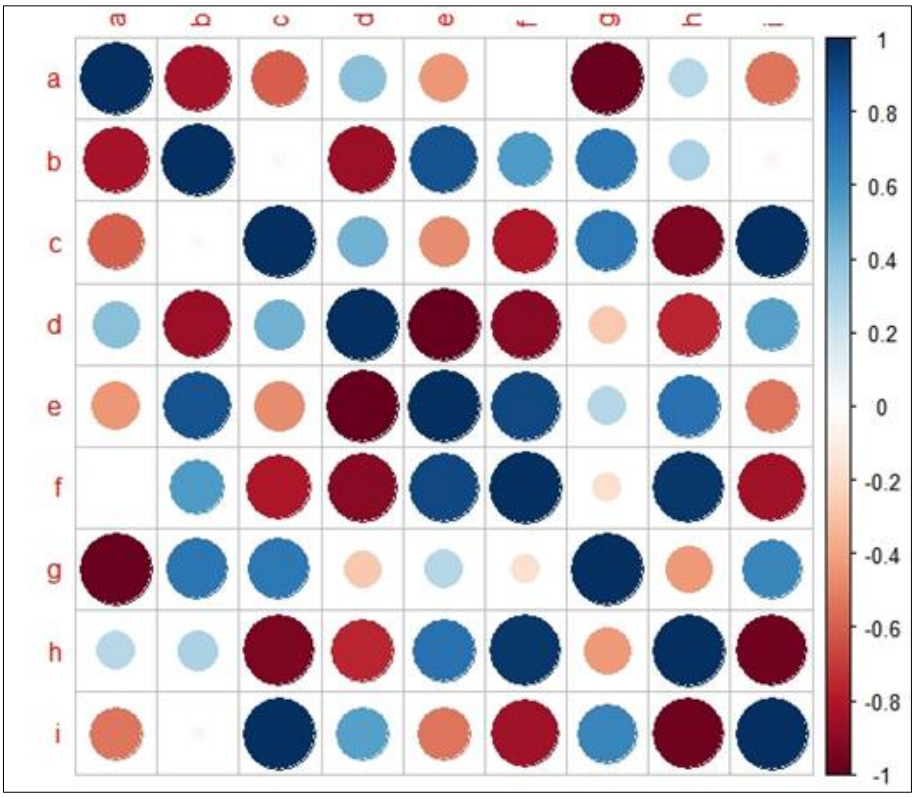
	p-Cymene	.gamma.-Terpinene	Cumic aldehyde	1,4-p-Menthadien-7-al	D-Limonene
Kishtwar	15.06	26.83	40.14	10.34	1.69
Bandipora	15.59	27.49	40.71	10.19	1.78
Pulwama	15.71	26.53	41.77	11.57	1.73

Fig 1: Figure showing essential oil composition in different populations of Kala zeera



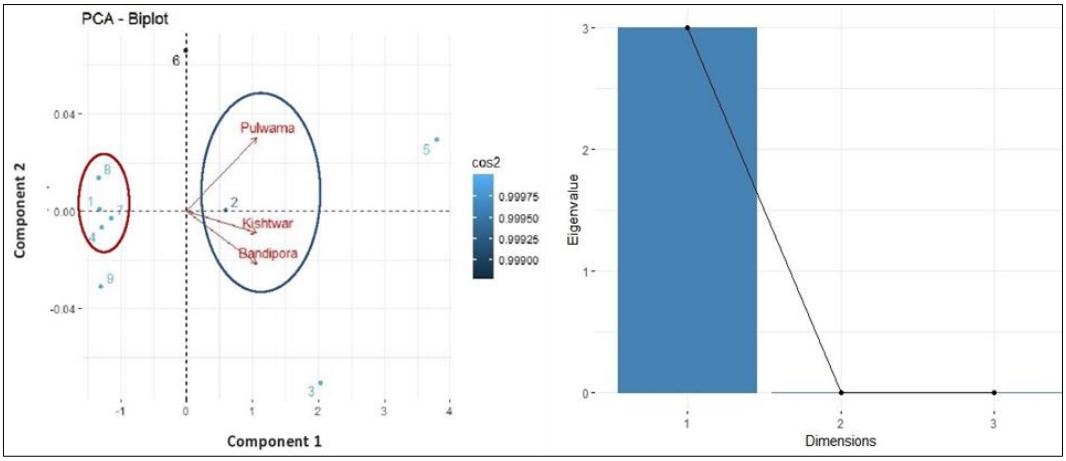
Where, a: Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)-; b: p-Cymene; c:.gamma.-Terpinene; d: 3-p-Menthen-7-al; e: Cumic aldehyde; f: 1,4-p-Menthadien-7-al; g: D-Limonene; h: Cyclooctasiloxane, hexadecamethyl-; i:.beta.-Myrcene
1: Kishtwar; 2: Bandipora; 3: Pulwama

Fig 2: Dendrogram showing the hierarchical clustering to interpret the distribution of 9 different essential oil compounds of Kala zeera



Where, 1: Bicyclo [3.1.0] hexane,4-methylene-1-(1-methylethyl)-; 2: p-Cymene; 3:.gamma.-Terpinene; 4: 3-p-Menthen-7-al; 5: Cumic aldehyde; 6: 1,4-p-Menthadien-7-al; 7: D-Limonene; 8: Cyclooctasiloxane, hexadecamethyl-; 9:.beta.-Myrcene

Fig 3: Pearson correlation analysis showing linear relationship between 9 different essential oil compounds of Kala zeera



Where, 1: Bicyclo[3.1.0] hexane,4-methylene-1-(1-methylethyl)-; 2: p-Cymene; 3:.gamma.-Terpinene; 4: 3-p-Menthen-7-al; 5: Cumic aldehyde; 6: 1,4-p-Menthadien-7-al; 7: D-Limonene; 8: Cyclooctasiloxane, hexadecamethyl-; 9:.beta.-Myrcene

Fig 4: Graphical representation of principal component analysis (PCA) showing the matrix of two variables a) Scatter plot between X-axis (PC1) and Y-axis (PC2) showing the distribution of 9 different compound and three temperate micro-climates of J&K, and b) Scree plot showing eigen values at Y-axis and number of factors/ components at X-axis

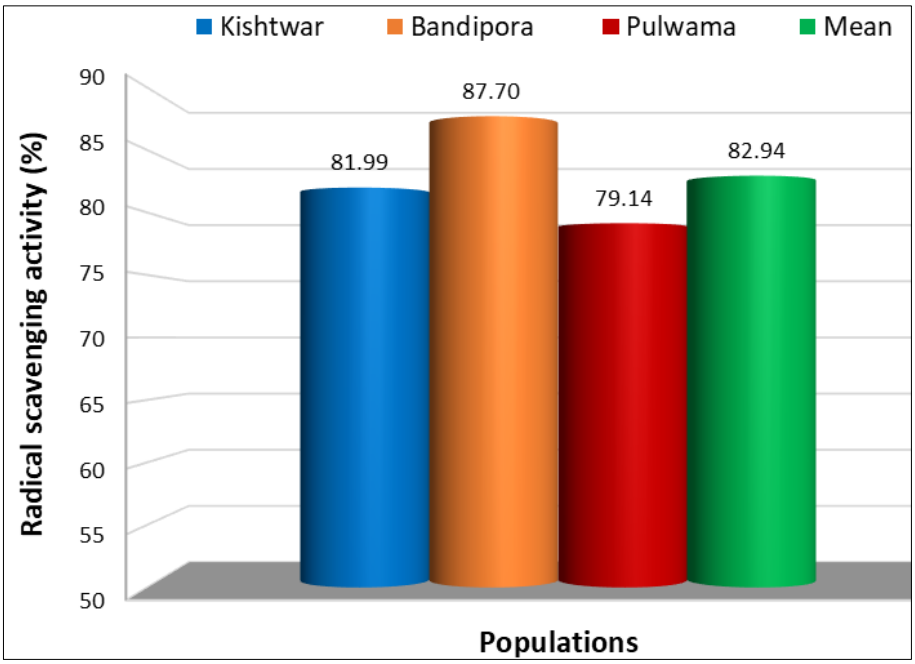


Fig 5: Graphical representation of antioxidant activity of Kala zeera populations

Conclusion

In this study, the variability of essential oil properties of different populations originated from different districts were evaluated. The considerable pharmacological attributes and the need for low input properties *B. persicum* in has made it desirable plant for domestication and cultivation. Present study showed a high level of diversity of the phytochemical traits among populations. The essential oil of *B. persicum* is considerable due to the antioxidant and antimicrobial activity and being safer than chemical counterparts, and this study represented a novel suggestion for the use of *B. persicum* global potentials to use in plant breeding programs.

Author's contribution

Conceptualization and designing of the research work (MHK, NAD, BAA); Execution of field/lab experiments and data collection (MHK, NAD, BAA, GHM, UF, MTA); Analysis of data and interpretation (MHK, NAD, AAL, SAD, AK); Preparation of manuscript (MHK, NAD, BAA, UF, SG, AHB).

Declaration

The authors should declare that they do not have any conflict of interest.

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