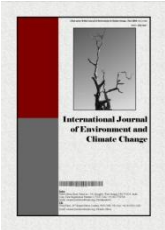

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Biochemical Characterization of Local Populations of *Physalis minima* L. Suggests Correlation between its Fruit and Medicinal Properties

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Physalis minima L. is known to possess numerous medicinal properties and has a potential to be utilized as a fruit plant. But the environment induced variations in biochemical constitution of this plant have not been identified. The present study was carried out for biochemical characterization of different populations of *Physalis minima* L. plants of Bihar and determine the correlation between medicinal and fruit value of the plant. Nineteen quantitative biochemical parameters were recorded for 70 plants of seven populations. Substantial variations, within and between populations, for all the biochemical characters was observed. The findings indicated the presence of positive and negative correlation within and between the nutritive and medicinal parameters of the plants. The biochemical characters were successfully able to distinguish the populations into different groups. The study thus concludes that the *P. minima* L. plants which have high medicinal value will also have highly nutritious fruits. It was also ascertained that the biochemical characters can be used as markers to characterize the plants and determine the relatedness between the plants of different regions. The information will thus enable easy selection of the commercially beneficial plants.

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Keywords: Physalis minima L.; secondary metabolites; principal component analysis; cluster analysis; correlation studies.

1. INTRODUCTION

The earliest agricultural practices involved altering the indigenous flora and exploiting plants beneficial for the humans. The sophistication of the agricultural practices and the abrupt increase in population lead to the demand of crops which can provide food security coupled with high production. This led to the narrowing of the genetic base and rapid decline in the genetic diversity of cultivated and wild plants. The rapid climate change has further aggravated the situation due to unpredictable and extreme weather conditions. The IUCN red list of 2018 gives a clear indication of this situation. The number of plants in the threatened category has increased from 5186 in 1996/1998 to 12564 in 2018 and in endangered category from 1197 in 1996/1998 to 4537 in 2018. In just one year 414 new species have been added in the endangered list (4123 in 2017 and 4537 in 2018). In India the situation is no different with 396 species of plants included in various lists of IUCN red 2018 [1,2]. Such a situation has caused an uproar in the scientific community, which is now again diverting its attention to exploit the natural diversity. As a result, recent years have seen an increasing amount of works concerning the use of minor crops and non-conventional fruits and vegetables, in order to identify plants suitable for a large range of environment. Among the large genetic diversity which can be utilized as non-conventional fruit, one plant *Physalis minima* L., belonging to the family Solanaceae has a great potential to be exploited as a fruit and a medicinal plant.

Physalis minima L. is an important indigenous plant of India belonging to the nightshade family Solanaceae*.* The members of this family are rich in secondary metabolites and include some of the important economical plants like tomato, potato, *Withania somniferum* and others. *P. minima* L. belongs to the genus *Physalis* consisting of 80-100 species, most of which are neotropical herbs. The genus gets its name from the Greek word '*Physalis'* which means "a bladder". It is a reference to the inflated, papery calyx characteristic of the members of *Physalis* [3,4]. The plant mostly grows as a cosmopolitan weed. It is a diploid plant which bears green fruits. The fruits are berries enclosed within enlarged, 10-ribbed, reticulately veined persistent calyx with slender and purplish rib [4].

The berry is a good source of vitamin C. The fruits have a good amount of protein, minerals, potassium, calcium, magnesium, iron and phosphorus [3]. The fruit has been used as decoration in culinary, ingredient for salads, desserts; exotic dressing of dishes in restaurants and as flavouring in jams and jellies. The plant particularly its fruit has gained importance in recent years due to its antioxidant and anticancer properties [5,6,7], though it has been widely used in Indian Traditional System of Medicines as diuretic, purgative, analgesic, anthelmintic, anti-inflammatory, antimicrobial, appetizer etc., since many centuries [8,9]. It has been found to contain many important constituents like steroids, withanoloides, flavonoids, terpenoids and others [10]. The plant has enormous medicinal values and a potential to be utilized as a fruit plant. Although, the medicinal value of the plant has been greatly explored, the fruit value of the plant is the least studied. The nutritive value of the plant particularly from Bihar, where it has widespread distribution as a broad-leaved weed, has furthermore not been explored in detail.

The plant is known as *ban tipariya* or *mako* in Bihar and is found growing in fields, along roadside and banks of rivers in wide range of soils but mostly well-drained, porous soil. The ripe fruits are savoured by the local populations [2,3]. The plant is also used for traditional medicinal purpose. Since, the plant occurs in a wide range of environment and the biochemical constitution of the plant is known to vary with different environmental conditions, the variations with respect to biochemical constitution is likely to be present. Thus, it is very important to identify the biochemical constitution of the plants growing in different regions for their proper utilization. Hence, the present study was carried out for biochemical characterization of different populations of the plant in Bihar, to identify the variability in the populations with respect to biochemical parameters and determine the correlation between medicinal and fruit value of the plant. The establishment of this relationship will help to identify variable population of plants which can be used as both fruit as well as medicinal plants.

2. MATERIALS AND METHODS

Physalis minima L. plants from the seven locations of Bihar (Badauna, Selao, Rajgir and

Harnaut regions of Nalanda district, two regions in Pusa of Samastipur district and Kurtha of Arwal district.) were assessed for its biochemical composition. Nineteen quantitative biochemical parameters were studied for all the plants of the seven populations. The observations were used to identify the relationship between the populations and correlation between fruit and medicinal value of the plant.

2.1 Experimental Material

The fruits, stem and leaves of the *Physalis minima* L. plants were examined to determine the amount of total soluble sugar, ascorbic acid, protein, total phenols, flavonoids and alkaloids content. A total of 70 fully mature plants, ten from each location were randomly selected. The leaves, stem and fruits of the selected plants were collected and bulked. The extracts of these were prepared in four solvents namely chloroform, ethanol, methanol and water using maceration method [10] with slight modifications. The experiments were conducted in three replications to validate the results. A total of 2520 samples were thus analysed in the experiments. Preliminary qualitative analysis was carried out for six phytochemicals and the samples which tested positive were used for further analysis.

2.2 Estimation of Nutritive Value of Leaves and Fruits

The nutritive value of the plant was assessed using fruits and leaves by estimating total soluble sugar, ascorbic acid and protein content. The protein content of the leaves and fruits was estimated by Folin-Ciocalteu (Lowry) method with some modifications [11]. The results were expressed in mg/g. The ascorbic acid (Vitamin C) content of the fruits and leaves of each of the seven populations was determined using 2,4 Dinitrophenyl hydrazine (DNPH) method. The amount of ascorbic acid was measured in µg ml^{-1} of the sample solution. The standard Anthrone method [11] with minor modifications was used for quantitative estimation of total soluble sugar content of fruits and leaves. The amount of total soluble sugar was measured in µg ml^{-1} of the sample solution.

2.3 Estimation of Medicinal Value of Leaves, Fruits and Stem

The amount of three secondary metabolites; total phenols, flavonoids and alkaloids; was determined to estimate the medicinal value of the plant. Fruits and leaves along with the extracts were used for the estimation of medicinal value. The total phenolic content of leaves, stem and fruits was estimated by analyzing the extracts using Folin-Ciocalteu method [12,13] with certain modifications. While whole fruit was used (TPF), the four extracts of stem (TPAS, TPES, TPMS AND TPES) and leaves (TPAL, TPEL, TPML AND TPEL) was used. The results were expressed in mg ml^{-1} as gallic acid equivalent. The alkaloid content and flavonoid content of the fruits and leaves was estimated by the methodology described by Harborne (1973) [14-16] with necessary modifications. The alkaloid content and flavonoid content were expressed in percent of the sample.

2.4 Statistical Analysis

The experiments were set up in a completely randomized design (CRD) with three replications for each treatment. All the data was analysed by running one way analysis of variance (one way ANOVA) using OP Stat software. The means were compared using Duncan's multiple range test to find the difference at 5% ($P<0.05$). The results were expressed as mean ± SE of three replications.

The data was further analysed using NTSYS-pc software [17]. The variability in the quantitative characters were identified by determining the standard deviations. The Pearson correlation coefficient between the pair wise combination of the biochemical characters was calculated using the formula

$$r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{n(\sum x^2) - (\sum x)^2}\sqrt{n(\sum y^2) - (\sum y)^2}}$$

Where, n is the number of pairs of data. The value of correlation coefficient ranges from -1 to +1.

2.5 Clustering of the Populations Based on Biochemical Characters

Sequential agglomerative hierarchical non-overlapping (SAHN) clustering method based on dissimilarity coefficients and taxonomic distances was used for tree building. The dissimilarity coefficients between the pair wise combination of the seven populations were calculated. The dendrogram was constructed based on dissimilarity coefficients by unweighted

paired group method using arithmetic mean (UPGMA).

Principal component analysis was also carried out to determine the relatedness between the populations. The contribution of the studied parameters towards two-dimensional ordination of the populations was determined.

3. RESULTS AND DISCUSSION

The ANOVA analysis results indicated the presence of substantial variations within and between populations for all the nineteen biochemical characters evaluated in the present study (Table 1 and 2). The total phenolic content of aqueous extract of leaves, protein content of leaves and total phenolic content of leaves showed very high differences between populations and had standard deviations greater than hundred. The amount of variation was highest for total phenolic content of aqueous extract of leaves. The total soluble sugar content of the leaves showed the least variations for the seven populations. The amount of the phytochemicals in each population is presented in Table 2.

3.1 Protein Content of Leaf (PCL) and Fruit (PCF)

The protein content of the fruits of *Physalis minima* L. was more or less similar for all the populations and was found to have an average value of 1135.31 mg/g for all the seven populations observed in the present study. The plants of UGH PUSA had the highest amount (1187.81 mg/g) of total protein in fruits and plants of Harnaut had the lowest value (1026.78 mg/g) (Table 2). The protein content of the leaves had an average value of 988.62 mg/g. The plants from Badauna had the highest amount (1152.23 mg/g) of protein in leaves and the plants from UGH PUSA had the lowest (822.83 mg/g) (Table 2).

The fruits have higher amount of protein than the leaves (1135.31 mg/g in fruits as compared to 988.62 mg/g in leaves). This may be due to the fact that the nutrients tend to accumulate in the fruits. The leaves on the other hand are mostly involve in the synthesis process [18]. While the fruits of all the regions were comparable for their protein content, the leaves had significantly different amount of protein as indicated by significantly different means of each of the populations. These differences can be attributed to the different climatic conditions of the region. It was observed by workers that the nutritional quality of the leaves is affected by the climatic changes [19]. The different genetic constitution of the plants of different regions can also contribute to these variations. However, since the amount of protein in fruits are similar, it can be concluded that the environment does not have much effect on the amount of protein in *P. minima* L. fruits. Thus, the fruits of the plant from different regions can be considered nutritionally similar for protein content.

3.2 Total Soluble Sugar of Leaf (TSSL) and Fruit (TSSF)

The fruits of *Physalis minima* L. plants were found to contain an average of 177.44 µg ml^{-1} of total soluble sugar while leaves had an average value of 172.7 µg ml^{-1}. The plants from Badauna had the highest amount (181.04 µg ml^{-1}) while that of NGH PUSA had the lowest amount of total soluble sugar in fruits. The plants of Selao had the highest amount (175.07 µg ml^{-1}), while the plants from UGH PUSA and NGH PUSA had the lowest amount (170.66 µgml^{-1}) of total soluble sugar in leaves (Table 2).

The leaves were found to have less amount of total soluble sugar than fruits. This observation can be attributed to the fact that though the sugars are synthesized in the leaves, they are stored as reserves in fruits and is thus responsible for the higher sugar content of the fruits [20]. It was particularly notable that the sweetness of the fruits was not correlated to the total soluble sugar of the fruits. This may be due to the presence of high amount of carbohydrates in the samples which is detected by Anthrone estimation but do not provide sweetness [21].

3.3 Ascorbic Acid Content of Leaf (AAL) and Fruit (AAF)

The ascorbic acid content of each of the seven populations of *Physalis minima* L. was found to have an average value of 144.54 µg ml^{-1} for fruits and 68.78 µg ml^{-1} for leaves. The ascorbic acid content of the fruits of the plants from Rajgir was highest (167.75 µg ml^{-1}). The ascorbic acid content of the leaves of the seven population was very variable and ranged from a highest value of 107.4 µg ml^{-1} for Selao to a lowest of 25.46 µg ml^{-1} for Kurtha. The lowest amount of ascorbic acid (105.9 µg ml^{-1}) was found to be present in the fruits of the plant from UGH PUSA (Table 2). Like other two nutritional component,

the ascorbic acid content of the leaves was much lower than the fruits.

The results of the investigation of the amount of the three nutritional components of the *P. minima* L. plant indicated that, a significant variation is present in the amount of these components among the plants of seven populations. These variations indicate the effect of environment on determining the nutritional benefit of the plant. The amount of each component was invariably higher in fruits that the leaves. The fruits are thus nutritionally superior than the leaves, as should be the case. The quantity of protein, ascorbic acid and sugar is appreciably high in fruits and leaves, which suggest the high nutritive value of the plant.

3.4 Total Phenol (TP) of Fruit, Stem and Leaves

The fruits of *Physalis minima* L. of all the seven populations were found to have an appreciable quantity of total phenols, with an average value of 195.71 mg ml^{-1} (Table 2). This value was greater than the amount of phenol in *P. peruviana* fruit [22], which is a similar fruit, commercially available in the market. The presence of higher amount of the phenols in *P. minima* L. fruits indicate that these fruits have higher medicinal value than the *P. peruviana* fruits.

The amount of total phenol in the leaves and stem of the plants was reliant on the solvent used for the preparation of extract as indicated by the amount of phenol present in each extract. The order of effectiveness of solvents to extract phenol from leaves was found to be chloroform > water > ethanol > methanol and for stem was observed to be water > ethanol > chloroform > methanol. An average value of 485.14 mg ml^{-1} total phenol for leaves and 158.95 mg ml^{-1} for stem was observed. The highest value was observed for aqueous extract of the leaves of Badauna (736 mg ml^{-1}) while lowest for methanolic extract of stem of Selao (81.17 mg ml^{-1}) (Table 2).

The amount of phenol is an indication of the curative properties of the plants. The presence of considerable quantity of total phenols in the samples thus indicates that the plant possesses medicinal properties. The order of plant parts, in terms of phenolic content, was found to be leaves > fruits > stem. This can be due to the fact that phenols are protective compounds which tend to be localized in the leaves of the plants to deter grazing animals and harmful insects [20]. The leaves thus prove to be better for medicinal use. The results indicated the presence of a large variations in the phenol content of the plants of different populations which may be due to the presence of genetic variability. The variability can also be attributed to the different climatic conditions of each region.

Table 1. The variability present in the nineteen quantitative biochemical parameters

S. No.	Variables	Mean	S.D.	Min	Max
1	Total soluble sugar-fruit (µg ml-1)	177.439	002.6136	0173.94	0181.04
2	Total soluble sugar-leaves (µg ml-1)	172.696	001.9575	0170.66	0175.07
3	Ascorbic acid- fruit (µg ml-1)	144.540	024.9211	0105.90	0167.75
4	Ascorbic acid-leaves (µg ml-1)	068.774	027.8723	0025.46	0107.40
5	Protein content-fruits (mg/g)	1135.303	051.3096	1026.78	1187.81
6	Protein content-leaves (mg/g)	988.611	118.3824	0822.83	1152.23
7	Total phenol-fruits (mg ml-1)	195.707	068.999	0132.30	0289.18
8	Total phenol-leaves (A) (mg ml-1)	537.674	120.839	0398.34	0736.54
9	Total phenol-leaves (E) (mg ml-1)	480.837	044.521	0428.31	0555.55
10	Total phenol-leaves (M) (mg ml-1)	315.734	033.192	0255.56	0354.58
11	Total phenol-leaves (C) (mg ml-1)	606.890	108.747	0480.96	0723.46
12	Total phenol-stem (A) (mg ml-1)	206.404	082.015	0110.27	0343.06
13	Total phenol-stem (E) (mg ml-1)	157.658	032.037	0134.85	0221.95
14	Total phenol-stem (M) (mg ml-1)	108.646	031.720	0081.17	0176.60
15	Total phenol-stem (C) (mg ml-1)	144.507	034.688	0093.27	0179.33
16	Flavonoids-fruits (%)	017.476	003.461	0013.74	0021.84
17	Flavonoids-leaves (%)	016.791	002.657	0013.60	0020.85
18	Alkaloids -fruits (%)	020.924	004.228	0016.67	0026.56
19	Alkaloids-leaves (%)	020.176	003.191	0016.14	0025.44

Table 2. Phytochemical constitution of seven populations

Phyochemical sample		Badauna	Harnaut	Selao	Rajgir	UGH - PUSA	NGH - PUSA	Kurtha	Mean	S.E (m)	C.D	C.V.
Total soluble sugar (µg ml^{-1})	F	181.04^{a}±0.95	175.19^{c} ±0.63	$179.09^{a,b}$±0.7	178.46^{b}±0.78	175.33^{c}±0.75	173.94^{c}±1.04	$179.02^{a,b}$±0.78	177.44	0.810	2.480	0.790
	L	173.79^{b}±0.15	170.69^{c}±0.33	175.07^{a}±0.5	$173.53^{b,c}$±0.3	170.66^{c}±0.3	170.66^{c}±0.3	$174.47^{a,b}$±0.39	172.7	0.334	1.024	0.335
Ascorbic acid (µg ml^{-1})	F	129.66^{d}±0.82	153.49^{c}±0.87	122.92^{e}±0.88	167.75a±1.18	105.9^{f}±0.38	$167.04^{a,b}$±0.92	165.02^{b}±0.72	144.54	0.851	2.606	1.020
	L	$84.48^{b,c}$±1.07	86.27^{b}±0.31	107.4^{a}±0.66	60.53^{d}±1.08	73.92^{c}±1.03	43.36^{e}±1.15	25.46^{f}±0.58	68.78	0.888	2.718	2.236
Protein content (mg/g)	F	1152.53^{a}±1.22	1026.78±0.95	1124.02±0.87	1152.23^{a}±1.17	1187.81±0.83	1152.87^{a}±0.7	1150.88^{a}±0.65	1135.31	0.935	2.862	0.143
	L	1152.23^{a}±1.17	1026.82^{c}±0.95	959.51^{d}±0.6	933.23^{e}±0.78	822.83^{g}±0.95	1120.73^{b}±0.82	904.93^{f}±0.93	988.62	0.902	2.762	0.158
Total phenol (mg ml^{-1})	F	133.91^{f}±1.83	289.18^{a}±0.6	132.3^{f}±1.12	145.82^{e}±0.44	271.4^{b}±1.75	155.35^{d}±1.39	241.99^{c}±1.63	195.71	1.349	4.130	1.194
	AL	736.54^{a}±0.74	572.93^{c}±1.98	650.48^{b}±0.39	453.94^{f}±1.46	$475.24^{d,e}$±1.44	398.34^{g}±1.56	476.25^{d}±2.12	537.68	1.498	4.586	0.482
	EL	523.75^{b}±1.29	451.18^{f}±1.31	456.49^{e}±1.04	555.55^{a}±0.7	484.68^{c}±0.86	465.9^{d}±1.78	428.31^{g}±0.95	480.84	1.174	3.597	0.423
	ML	336.02^{c}±0.81	255.56^{g}±1.54	300.31^{f}±0.99	343.23^{b}±1.73	307.58^{e}±1.86	312.86^{d}±0.22	354.58^{a}±1.02	315.74	1.281	3.923	0.703
	CL	480.96^{f}±1.07	512.4^{d}±1.26	500.61^{e}±1.5	610.77^{c}±1.14	723.46^{a}±1.01	710.23^{b}±1.31	$709.8^{b,c}$±1.7	606.89	1.302	3.987	0.372
	AS	169.49^{e}±1.71	110.27^{f}±1.03	183.11^{c}±1.49	$166.24^{e,f}$±2.12	176.61^{d}±1.15	343.06^{a}±1.56	296.05^{b}±1.63	206.41	1.558	4.771	1.307
	ES	141.65^{d}±1.05	221.95^{a}±1.17	151.86^{c}±1.47	134.85^{e}±1.06	$138.82^{d,e}$±0.78	136.5^{e}±0.7	177.96^{b}±0.57	157.66	1.007	3.084	1.106
	MS	$107.08^{b,c}$±1.74	86.28^{e}±1.15	81.17^{f}±0.73	109.27^{b}±0.57	102.67^{c}±1.68	176.6^{a}±1.43	97.45^{d}±0.85	108.65	1.236	3.786	1.971
	CS	136.25^{d}±1.02	167.35^{b}±1.33	151.24^{c}±1.65	93.27^{f}±1.63	105.08^{e}±1.02	$179.03^{a,b}$±1.27	179.33^{a}±0.58	144.51	1.258	3.854	1.508
Flavonoids (%)	F	$17.34^{b,c}$±0.61	14.77^{c}±0.63	18.77^{b}±0.92	14.1^{c}±0.64	$21.77^{a,b}$±0.5	13.74^{c}±0.57	21.84^{a}±1.48	17.48	0.821	2.516	8.143
	L	$18.24^{b,c}$±0.95	$16.07^{c,d}$±0.7	20.85^{a}±0.73	18.64^{b}±0.54	16.44^{c}±0.18	13.6^{d}±0.31	13.7^{d}±0.53	16.8	0.610	1.869	6.297
Alkaloids (%)	F	24.5^{b}±1.21	$24.96^{a,b}$±0.8	26.56^{a}±0.43	18.74^{c}±0.35	$17.96^{c,d}$±0.2	$17.08^{c,d}$±0.39	16.67^{d}±0.19	20.93	0.611	1.872	5.060
	L	25.44^{a}±1.1	20.6^{c}±0.47	$19.44^{c,d}$±0.53	16.14^{d}±0.39	$18.87^{c,d}$±0.49	17.67^{d}±1.21	23.07^{b}±0.5	20.18	0.733	2.246	6.296

F-Fruits, L-Leaves, AL-Aqueous extract leaves, EL-Ethanolic extract leaves, ML-Methanolic extract leaves, CL-Chloroform extract leaves, AS-Aqueous extract stem, ES-Ethanolic extract stem, MS-Methanolic extract stem, CS-Chloroform extract stem. Values expressed as mean ± SE. Mean value in rows bearing same letter are not significantly different using Duncan's Multiple Range Test at 5% level

Table 3. Pearson correlation coefficient among the biochemical traits
Critical values of Pearson's coefficient df =5, 0.755 (p= .05), 0.875 (p= .01)

	TSSF	TSSL	AAF	AAL	PCF	PCL	TPF	TPAL	TPEL	TPML	TPCL	TPAS	TPES	TPMS	TPCS	FF	FL	AF
TSSL	0.8941*	*																
AAF	-0.1294	0.0102																
AAL	0.1880	0.0957	-0.6626															
PCF	0.1954	0.1806	-0.2484	-0.3560														
PCL	0.0828	-0.0668	0.2777	0.1002	-0.2641													
TPF	-0.4932	-0.5394	-0.0888	-0.1701	-0.3461	-0.4833												
TPAL	0.6648	0.4556	-0.4985	0.7258	-0.2627	0.3766	-0.2781											
TPEL	0.3129	0.0772	-0.0306	0.1710	0.3369	0.1342	-0.4962	0.1101										
TPML	0.5757	0.5658	0.2372	-0.5780	0.7482	-0.0989	-0.4348	-0.1498	0.3447									
TPCL	-0.5027	-0.3621	0.2057	-0.7837*	0.5642	-0.4612	0.3312	-0.8712	* -0.2143	0.3522								
TPAS	-0.2098	0.0053	0.4340	-0.7539	0.4599	0.1490	-0.2016	-0.5496	-0.3666	0.4667	0.6714							
TPES	-0.1776	-0.1643	0.2249	0.0745	-0.8738*	-0.0045	0.6689	0.1385	-0.5818	-0.5843	-0.2786	-0.3202						
TPMS	-0.4884	-0.4499	0.4137	-0.5194	0.3509	0.4687	-0.3043	-0.5505	0.1017	0.1858	0.5028	0.7229	-0.4626					
TPCS	-0.1928	-0.0242	0.3867	-0.2820	-0.4201	0.4199	0.1399	-0.0109	-0.8116*	-0.2142	0.0231	0.5287	0.5174	0.2237				
FF	0.2801	0.2987	-0.5588	-0.0754	0.4199	-0.6294	0.3627	0.1158	-0.3962	0.2794	0.3037	0.0724	-0.0234	-0.4636	-0.0351			
FL	0.5387	0.5106	-0.4892	0.8210*	-0.0415	-0.0613	-0.4975	0.6343	0.4671	-0.0889	-0.7078	-0.6543	-0.2425	-0.5470	-0.5420	-0.0419		
AF	0.3597	0. 7570*	-0.3934	0.8898*	*-0.5887	0.3449	-0.2047	0.8657*	0.0025	-0.5312	-0.9496	-0.6642	0.3324	-0.5567	0.0552	-0.1557	0.7055	
AL	0.5749	0.3386	-0.2074	0.0498	-0.0844	0.3379	0.0746	0.6785	-0.2228	0.1761	-0.3621	-0.0637	0.2865	-0.3210	0.3521	0.4105	-0.0711	0.3261

TSSF: Total soluble sugar-fruit (µg ml-1); TSSL :Total soluble sugar-leaves (µg ml^{-1}); AAF: Ascorbic acid- fruit (µg ml^{-1}); AAL : Ascorbic acid-leaves (µg ml^{-1}); PCF: Protein content fruits (mg/g); PCL: Protein content leaves (mg/g); TPF: Total phenol fruits (mg ml^{-1}); TPAL: Total phenol leaves (A-Aqueous extract) (mg ml^{-1}); TPEL: Total phenol leaves E-(Ethanolic extract) (mg ml^{-1}); TPML: Total phenol leaves (M-Methanolic extract) (mg ml^{-1}); TPCL: Total phenol leaves (C-Chloroform extract) (mg ml^{-1}) TPAS: Total phenol stem (A) (mg ml^{-1}) TPES: Total phenol stem (E) (mg ml-1); TPMS: Total phenol stem (M) (mg ml^{-1}); TPCS: Total phenol stem (C) (mg ml^{-1}); FF: Flavonoids fruits (%); FL: Flavonoids leaves (%); AF: Alkaloids fruits (%); Alkaloids leaves (%)
**Significant at p=0.05; ** Significant at p=0.01*

Table 4. Contribution of the principal components to the variations in the nineteen biochemical quantitative characters

i	Eigenvalue	Percent	Cumulative
1	6.508304	34.2542	34.2542
2	4.316875	22.7204	56.9746
3	3.003653	15.8087	72.7833
4	2.850399	15.0021	87.7854
5	1.377086	7.2478	95.0332

3.5 Alkaloid Content of Fruit (AF) and Leaves (AL)

The alkaloid content of *Physalis minima* L. was significantly variable and had an average value of 20.93 % for fruits and 20.18 % for leaves. The plants of Selao had the highest amount (26.56 %) while the plants from Kurtha were found to have the lowest amount (16.67 %) of alkaloids in fruits. The alkaloid content of the leaves was comparable to the fruits with only a minor difference with a range of highest value of 25.44 % for Badauna to a lowest of 16.14 % for Rajgir. This may be due to the fact that the alkaloids are present in leaves and fruits as a protective agent [20]. However similar findings were obtained and it was observed that the alkaloid content of leaves was highest in Nigerian softwood [15]. The presence of alkaloids in the samples can directly be correlated to the antimicrobial properties of the plant [23,10]. The results thus suggest the medicinal properties of the plant. The alkaloid content of the plant showed variations among all the seven populations, which may be due to genetic reasons or the effect of the environment.

Table 5. Contribution of the nineteen biochemical quantitative characters to the first three principal components

Character	PC1	PC2	PC3
TSSF	0.5652	0.5939	0.0883
TSSL	0.4175	0.5506	0.1128
AAF	-0.4998	-0.1521	-0.5044
AAL	0.8812	-0.0974	-0.043
PCF	-0.4023	0.8281	0.2356
PCL	0.1537	-0.1039	-0.84
TPF	-0.2098	-0.6819	0.6137
TPAL	0.9083	0.0618	-0.0416
TPEL	0.2057	0.6412	-0.3547
TPML	-0.3194	0.8189	0.0852
TPCL	-0.9265	0.1065	0.3372
TPAS	-0.79	0.1657	-0.1505
TPES	0.2054	-0.8689	0.1604
TPMS	-0.7175	0.1137	-0.5935
TPCS	-0.2226	-0.5783	-0.1869
FF	0.0104	0.1835	0.9133
FL	0.8291	0.3853	-0.0407
AF	0.9354	-0.238	-0.1846
AL	0.3713	-0.0379	0.1872

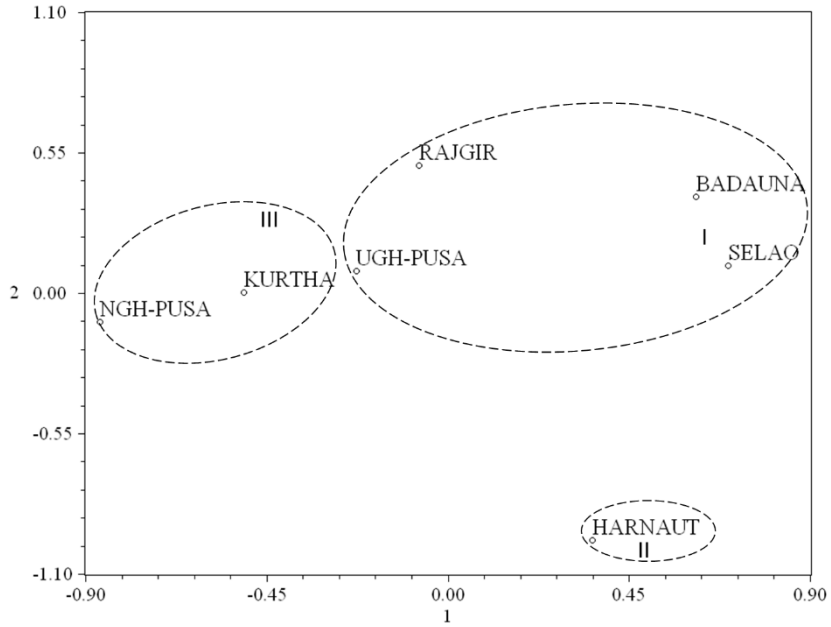

Fig. 1. Two-dimensional ordination of the seven populations by Principal component analysis based on nineteen biochemical characters

Table 6. Dissimilarity coefficients based on nineteen quantitative biochemical characters for pair-wise combinations between the seven populations

	Badauna	Harnaut	Selao	Rajgir	Ugh-pusa	Ngh-pusa
Harnaut	1.4982					
Selao	0.8755	1.2838				
Rajgir	1.2098	1.5905	1.2211			
UGH-PUSA	1.4742	1.4903	1.3144	1.1694		
NGH-PUSA	1.6694	1.6299	1.7249	1.3229	1.4247	
Kurtha	1.485	1.5894	1.482	1.3963	1.2638	1.3014

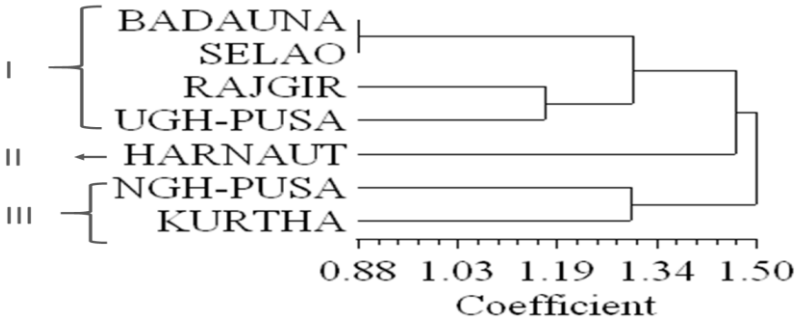

Fig. 2. Dendrogram based on average taxonomic distance for nineteen quantitative biochemical characters

3.6 Flavonoid Content of Fruit (FF) and Leaves (FL)

The flavonoid content of the fruits of the seven populations was comparable, and ranged from a highest value of 21.84 % for Kurtha to a lowest of 13.74 % for NGH PUSA with an average value of 17.48 %. The leaves of *Physalis minima* L. plants were found to have an average value of 16.8 % flavonoids for all the seven populations. The plants from Selao had the highest amount (20.85 %) of flavonoid content in leaves. The leaves of the plants from NGH PUSA had the lowest amount (13.6 %) of flavonoids. The leaves of *Physalis minima* L. possess less flavonoids as compared to the fruits. Since presence of flavonoids indicate the medicinal value, fruits can be considered better for medicinal use.

The results for all the three secondary metabolites, showed that the plant contains significant value of secondary metabolites in leaves, stem and fruits. Hence, the plant has ability to be used as medicinal plant. The fruits were found to contain more amount of each of the component as compared to leaves and stem. Thus, fruits are the best component for medicinal use. Since, fruits also have high nutritive value, it can be concluded that the direct consumption of the *P. minima* L. fruit can provide both nutritional and therapeutic advantages. The results confirm the opinion of many workers who have highlighted the medicinal importance of the fruits of *Physalis* species [24,25].

3.7 Correlation between the Biochemical Characters

The correlation studies between biochemical parameters can be used to determine the interrelationship between fruit and medicinal value of fruit, leaves and stem [26]. The Pearson correlation coefficients computed for the pair wise distribution of the nineteen biochemical characters suggested a correlation between the characters. The value of correlation coefficient ranged from -0.9496 to 0.8941 (Table 3). The biochemical characters were either positively or negatively correlated. The significant positive correlation in decreasing order was present between the pair TSSF and TSSL (0.8941), AF and AAL (0.8898) and AF and TPAL. The character pairs AL and TPCL (-0.9496), TPES and PF (-0.8738), TPCL and TPAL (-0.8712), TPEL and TPCS (-0.8116) and TPCL and AAF (-0.7837) had consecutively significant negative correlation. All the other character pairs were either positively or negatively correlated but the values were not statistically significant at 5 %.

The findings indicate that the correlation is present within and between the nutritive and

medicinal parameters of the plants. A certain amount of negative correlation is present between the characters, particularly total phenol content of extracts in different solvents. These findings indicate the distinct ability of the solvents in extraction of phenols from different samples. The positive correlation between total soluble sugar of leaves and fruits indicates that, the amount of sugar stored in fruits is directly related to the amount of sugar produced in leaves. The results also indicate that the alkaloid and phenol contents are correlated to ascorbic acid content. These findings clearly indicate that the nutritive and medicinal value of the plant are directly correlated.

3.8 Principal Component Analysis and Spatial Distribution of the Populations

Principal component analysis revealed that the first five principal components accounted for 95 percent of the variations present in the nineteen biochemical characters of the seven populations evaluated in the present study (Table 4). The insight into the individual contribution of each of the characters to the first three principal components showed that most of the characters significantly contributed towards the first principal component, eight characters significantly contributed towards second principal component and only five characters contributed towards the third principal component (Table 5). The contribution of AF towards first principal component had the highest positive value, followed consecutively by TPAL, AAL, FL, TSSF and TSSL. The TPCL had the highest negative contribution to the first principal component followed by TPAS, TPMS and AAF. Other parameters had less contribution towards first principal component. The parameter PCF followed by TPML, TPEL, TSSF and TSSL had the highest positive contribution while TPES followed by TPF and TPCS had the highest negative contribution towards the second principal component. The FF which had negligible contribution towards first two principal components, had highest positive contribution to third principal component followed by TPF. The PCL had highest negative contribution to the third principal component followed by TPMS and AAF. The results indicated that the parameters determining medicinal properties had higher contribution towards first principal component while the parameters for nutritive value had more contribution towards second principal component. Thirteen parameters namely AAL, PCF, PCL, TPF, TPAL, TPML, TPCL, TPES, TPAS, TPMS, FF, FL and AF had comparatively higher contribution towards first three principal components. These parameters can thus be used as markers to identify diversity among the *P. minima* L. plants.

Principal component analysis based two-dimensional ordinations of the seven populations along the two axes, based on nineteen biochemical traits, separated the populations into three groups (Fig. 1). The first group consisted of Badauna, Selao, Rajgir and UGH PUSA, Harnaut was present in second group. The third group consisted of NGH PUSA and Kurtha. The findings indicated the presence of variations and distinctiveness among each population. The grouping of the plants of different districts in different groups indicates that the climatic conditions play a role in determining the biochemical composition of the *P. minima* L. plant. However, the distance between the groups is not large, which suggest a minor role of the environment in determining the biochemical profile of the plant. The plants from Nalanda district were placed in the same group, which shows that these plants are more similar to each other in terms of their biochemical composition. It also suggests that same environmental conditions, lead to similar biochemical profile. However, plants from Samastipur district were placed in different groups, which also suggest that genetic constitution of the plant determines the biochemical profile of the plant. Since the plants from Harnaut were present in single group, they were most distinct than the plants from other regions. The findings suggest a combined role of the environment and genetic constitution in determining the amount of the biochemicals present in the plant. Hence, it can be inferred that the nutritive and medicinal value of the *P. minima* L. plant depends upon its genetic makeup and the region in which it is growing. The results suggested that the biochemical characters evaluated in the present study among seven populations, showed a significant variation within and between the seven populations, which can be used to group the populations.

3.9 Dissimilarity Coefficients Based on Pair Wise Combinations of the Seven Populations

The dissimilarity coefficients in the form of average taxonomic distances based on nineteen quantitative biochemical characters for pair wise combinations between the seven populations

ranged from 0.8755 to 1.7249 (Table 6). The highest value of the dissimilarity coefficient was observed between the population pair Selao and NGH PUSA while the lowest dissimilarity was observed between Badauna and Selao. Only the population pair Selao and Badauna had a dissimilarity coefficient less than one suggesting that the two populations were biochemically most similar. Other populations had values higher than one. The Harnaut was found to be the most distinct population among the seven populations based on biochemical characters.

3.10 Cluster Analysis Based on Nineteen Quantitative Biochemical Characters using UPGMA

The biochemical characters-based clustering of the seven populations by unweighted paired group method using arithmetic mean (UPGMA) based on taxonomic distances identified three principal clusters at 25 phenon level.

The first cluster consisted of four populations Badauna, Selao, Rajgir and UGH PUSA. The two biochemically most similar populations Badauna and Selao were present in this cluster. The second cluster was mono-genotypic and had the population Harnaut segregated from other populations. The populations Kurtha and NGH PUSA were present in the third cluster (Fig. 2).

4. CONCLUSION

The populations evaluated in the present study showed significant variations in their phytochemical constitution, which indicates the presence of high biochemical diversity among the *Physalis minima* L. plants of Bihar. The biochemical characters estimated were successfully able to distinguish the populations into different groups. These biochemical characters can thus be used as markers to characterize plants of different regions and determine the relatedness between them. The estimation revealed the presence of a high amount of total soluble sugar, ascorbic acid, protein, phenols, flavonoids and alkaloids in leaves and fruits of the plants of the seven populations. The biochemical profile clearly highlights the nutritive and medicinal value of the fruit of the plant. The plant can thus, be exploited as a non-conventional fruit having medicinal value. This study will therefore help to establish the status of the plant in commercial market.

The correlation studies revealed a significant correlation within and between the parameters evaluated for nutritive and medicinal value. The correlation between the fruit and medicinal properties of the plant was particularly apparent. Hence, the results suggests that the *P. minima* L. plants which have high medicinal value will have highly nutritious fruits. However, the further validation of the results will strengthen the findings of the present work. The information will thus enable easy selection of the commercially beneficial plants.

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COMPETING INTERESTS

The authors wish to express that there is no conflict of interest for the article entitled “Biochemical characterization of local populations of *Physalis minima* L. suggests correlation between its fruit and medicinal properties.”

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