# The Study of Morphological Characteristics of St. John's Wort (*Hypericum perforatum* L.) Populations in Iran's Natural Habitats

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## Abstract

**Background: St. John's wort (*Hypericum perforatum* L.) is an important medicinal plant that has been widely used for its antidepressant properties.**

**Objective: In this study morphological characteristics variation of populations of *H. perforatum* were investigated.**

**Methods: In this study, different morphological characteristics of 25 *H. perforatum* populations in Iran's natural habitats were evaluated in 2010.**

**Results: The results indicated that the highest coefficients of variations were found in flowers number, the fifth internodes' length and capsule length (respectively, 93.46, 85.28 and 84.7%); however the lowest coefficients of variations were seen in flower width (11.72%) and sepal length (11.91 %). The largest dimensions of flowers, sepals and petals related to the population which was gathered from Zanjan/Tarum city. The highest leaves dimensions and dark glands density on the bottom leaves were observed in Alamut and Kalaleh populations. The highest positive significant correlations were seen between flower length with flower width; dark glands density on the middle leaves surface area with dark glands density on the bottom leaves surface area; petal length with flower length and width; capsule length with capsule width; non-flowering branches number with branches number; light glands density on the middle leaves surface area with light glands density on the bottom leaves surface area. The lowest significant correlations were observed between capsule length with flower length and width. The principal components analysis showed that four components explained 68.9 % of total variance. The cluster analysis divided these populations into four clusters with no consistency in their geographical distributions.**

**Conclusion: In conclusion, this study indicated that there were high variations among the Iranian St. John's wort populations which can be utilized in the breeding programs.**

**Keywords: *Hypericum perforatum* L., Iran, Population, Morphological characteristics**

## Introduction

Hypericum is a genus of Hypericaceae or Clusiaceae family which has 17 species in Iran but only one species *H. perforatum* L. has high medicinal value. This species grows widely in the north, north-west, west and northeast Iran, particularly in Ardebil, Astara, Gorgan, Kohgiluyeh and Boyr-Ahmad, Fars provinces and in rangeland and mountain hills of Alborz [12]. This plant has a perennial habit, slow and creeping nature in the first year and flowering from the second year [13, 17, 21].

Iran's natural habitat is as inheriting source for provision and production of medicinal herbs under field conditions. There are many factors like origin, genotype, nutrient elements, climate and soil which are influencing the quantity and quality of plants [11]. In this order, the previous studies were found that the altitude had significant effect on growth and the secondary metabolism of St. John's wort and flavonoid content decreased as altitude increased. Also, the amount of tannins, ascorbic acid and cartenoid differed in a narrow range [22]. Nonetheless, 1250 meters above sea level and more than 760 mm of rainfall are the best conditions for this plant in natural habitat, but it can grow at higher elevation as well. Of course, elevations more than 1500 meters above sea level and lowlands condition reduce the seedling growth due to sever cool condition and less than 500 mm rainfall respectively [11 ,12 , 22].

However, St. John's wort is an important plant because of it's worthily compounds such as hypericin, pesodohypericin, porotohypericin, porotosiclohypericin and hyperforin. Hypericin is a key compound for evaluation of the quality of this plant. These compounds are available in dark glands on the leaves, stems, flowers and stamens of the plant [10, 15, 16]. Several clinical studies have demonstrated the effectiveness of St. John's wort extracts as a phytomedicinal treatment for mild to moderate depression due to its antidepressant properties. Proven photodynamic, antiviral, antiretroviral, and antitumor activities of *Hypericum* extracts also suggest use of this plant in HIV and cancer treatments [9, 12, 19]. For this reason, this plant has been introduced into many regions of the world [5, 19]. However, on the aspect of St. John's wort morphological populations in Iran, no comprehensive assessment has been done yet. Therefore, with regard to the great dispersal of this plant in Iran, any investigation on St. John's wort morphological populations would be very helpful in optimization of the production and performance of this plant quantitatively and qualitatively. The main objectives of our research were: (1) evaluation of morphological characteristics variation among populations; (2) classifying of populations based on morphological traits; (3) determining the effect of geographical distributions on the morphological traits of populations; (4) determining the possible correlations between morphological traits; (5) identifying characteristics which could be suitable descriptors in future breeding programs.

## Materials and Methods

Totally twenty-five populations of St. John's wort (*Hypericum perforatum* L.) were collected from different areas of Iran in the spring and summer 2010 (Figure 1). Geographical origins of twenty-five St. John's wort populations are listed in Table 1. All Plants samples were collected at flowering stage.

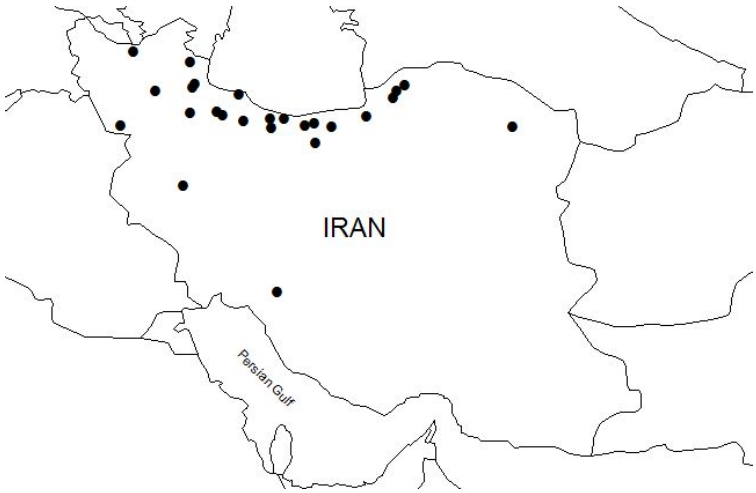


**Fig. 1- The geographical origins of *H. perforatum* populations**

**Table 1- The geographical origins and climatic conditions of *Hypericum perforatum* L. populations**

| Populations no. | Region originated | Altitude (m) | Latitude, N | Longitude, E | Climate [A] |
|---|---|---|---|---|---|
| 1 | Firooz Kooh road to Gadok | 1902 | 35°52'36. 3 " | 52°57'29. 6 " | semi-arid, temperate |
| 2 | Pol-e Sefid to Sangdeh | 1476 | 36°04' 42.8 " | 53°10'10. 5 " | semi-arid with hot summer and winter relatively cool |
| 3 | between Shirgah and Zirab | 322 | 36°15'56. 0" | 52°53'40. 9 " | semi-wet with hot summer and winter relatively cool |
| 4 | Gorgan– Ziyarat | 1188 | 36°40'53. 1" | 54°27'54. 7" | semi-arid, temperate, hot |
| 5 | Azadshahr to Shahrood | 455 | 37°01'51. 3" | 55°16'58. 0" | semi-arid , temperate, hot |
| 6 | between Kalaleh and Dahaneh village | 202 | 37°24'50. 5" | 55°29'35. 1" | semi-arid , temperate, hot |
| 7 | Siahbisheh | 558 | 36°09'43. 0" | 52°21'26. 0" | semi-wet with temperate summer and cool winter |
| 8 | Asara to Karaj | 1846 | 36°02'24. 8" | 51°11'13.8" | semi-arid with relatively hot summer and relatively cool winter |
| 9 | Jirandeh-Damash-Pakdeh | 1581 | 36°44'00. 0" | 49°47'46. 2" | semi-arid, cool, temperate |
| 10 | Macrowave station – west Rustam Abad | 514 | 36°54'08. 9" | 49°01'06. 9" | arid , temperate, cool |
| 11 | Lahijan to Astaneh | 20 | 37°12'43. 0" | 50°10'10. 5" | semi-wet, temperate, cool |
| 12 | Talesh | 53 | 37°47'09. 4" | 48°54'34. 4" | semi-wet, temperate, cool |
| 13 | 20km to Namin | 966 | 38°23'46. 7" | 48°36'15. 3" | border between dry and semi-wet, temperate, cool |
| 14 | Asalem to Khalkhal | 1829 | 37°37'35. 1" | 48°46'39. 0" | semi-wet, temperate, cool |

**Continue Table 1- The geographical origins and climatic conditions of *Hypericum perforatum* L. populations**

| Populations no. | Region originated | Altitude (m) | Latitude, N | Longitude, E | Climate [A] |
|---|---|---|---|---|---|
| 15 | Kalardasht | 2238 | 36°31'15. 7" | 51°05'53. 9" | semi-wet, temperate, cool |
| 16 | Noor | -3 | 36°34'14. 7" | 51°52'54. 4" | semi-arid with hot summer and relatively cool winter |
| 17 | Alamut-45km Qazvin | 1573 | 36°25'12. 2" | 50°15'31. 1" | semi-wet, temperate, cool |
| 18 | Zanjan-Tarum | 2119 | 36°52'16. 5" | 48°39'12. 4" | Temperate, semi-arid |
| 19 | Nahavand, Sarab(Gian forest) | 1669 | 34°09'10. 5" | 48°13'26. 5" | semi-wet, temperate, cool |
| 20 | Sisakht to Mishi spring | 2329 | 30°51'35. 9" | 51°28'40. 3" | semi-wet, temperate, cool |
| 21 | Baneh (30 km from Saghez) | 1581 | 36°10'13. 0" | 46°03'53. 3" | semi-wet, temperate, cool |
| 22 | Asheghlo to Vaniagh | 1294 | 38°56□02. 0" | 46°46'10. 7" | arid, temperate, cool |
| 23 | Between Hashtrod and Mianeh | 1861 | 37°25'54. 0" | 47°20'20. 2" | arid, temperate, cool |
| 24 | Chenaran, Maravetapeh | 754 | 37°45'01. 9" | 55°54'32 .3" | semi-arid, temperate, hot |
| 25 | Dizbadolia- Neyshabur | 1738 | 36°05' 59.4 " | 59°17'14. 4" | arid, temperate, cool |

[A] Yearly mean temperature in warm, temperate and cool climates are 15 – 25 8C, 10 – 15 8C and 0 – 5 8C. Yearly mean, respectively. Rainfalls in semi-humid, semi-arid and arid climates are 600 – 1400 mm, 300 – 600 mm and 100 – 300 mm, respectively

To study the botany traits (microscopic and macroscopic characteristics) of St. John's wort plant, 10 plant samples of each population in each region were randomly collected. The macroscopic traits included stem height; number of branches; the smallest and largest secondary stems length; number of stem internodes; the fifth internodes' length; stem diameter; numbers of flower and non-flower branches on the main stem; the bottom and top leaves dimensions; inflorescence, sepal and petal dimensions; numbers of flowers and capsules on main stem and flower diameter before opening. Microscopic characteristics included dark and light glands density on the leaves (the bottom, middle and top leaves), dark gland density on the petal, and dark and light glands density on the leaves surface area. The macroscopic and microscopic characteristics including dark glands density on the leaves and petal were measured at the collection sites. The other microscopic traits were examined at the laboratory.

Data were analyzed with SAS 6.12 and SPSS 16.0 software. Simple statistics (i.e. mean, maximum, minimum and coefficient of variation) were used in order to compare genetic variation in pre-planting stages. Statistical analysis included variance analysis, simple and Pearson correlation coefficient and component and cluster analysis on all morphological traits. Also, the means of results were compared by Duncan's multiple range tests. The cluster analysis was shown as a dendrogram indicating the estimated relations among St. John's wort populations. The dendrogram was created by UPGMA (using unweighted pair group method using arithmetic averages).

# Results

The populations of St. John's wort were significantly different in all studied parameters ($p<0.05$). St. John's wort populations were evaluated in respect of dark and light glands density on the leaves and dark glands density on the petal. Kalaleh population had the greatest dark glands density on the leaves i.e. 58.54, 99.9 and 93.2 glands on the top, middle and bottom leaves, respectively. The least dark glands density on the leaves was seen in Baneh population with 3.1, 14.9, 18.8 glands on the top, middle and bottom leaves, respectively. The greatest and least dark glands density on the petal were observed in Lahijan (28.1 glands) and Nahavand (12.9 glands) populations, respectively. The greatest light glands density were recorded on the top leaves in Siahbisheh population (390.9 glands), on the middle leaves of Macrowave population (478 glands) and on the bottom leaves Chenaran population (497.7 glands). However, the least light glands density were seen on the top leaves in Pol-e Sefid population (59.2 glands) and on the middle and bottom leaves (respectively, 49.6 and 14.6 glands) in Khalkhal population (Table 2).

**Table 2- Analysis of variances for the morphological traits of *H. perforatum* L. populations**

| Traits | Mean Square | | | | Range variation | | Means traits | |
|---|---|---|---|---|---|---|---|---|
| Source variation | Coefficient of Variation (CV)% | Error | Populations | Repeat | Min | Max | Min | Max |
| Df | - | 216 | 24 | 9 | - | - | - | - |
| Length stem | 19.14 | 102.73 | 2763.25$^{**}$ | 110.90$^{ns}$ | 20.3 | 100 | 29.99 | 85.56 |
| Number of branches | 18.17 | 41.64 | 416.22$^{**}$ | 26.54$^{ns}$ | 17 | 62 | 24.9 | 43.2 |
| The smallest secondary stem length | 80.56 | 1.48 | 7.79$^{**}$ | 0.95$^{ns}$ | 0.1 | 4.8 | 0.68 | 4.51 |
| The largest secondary stem length | 67.64 | 36.73 | 164.23$^{*}$ | 21.79$^{ns}$ | 1.2 | 33 | 3.89 | 21.46 |
| Stem diameter | 30.56 | 0.91 | 5.82$^{**}$ | 1.36$^{ns}$ | 1.5 | 7 | 1.85 | 4.8 |
| Number of stem internodes | 20.04 | 18.40 | 108.29$^{**}$ | 5.07$^{ns}$ | 10 | 39 | 12.7 | 25.4 |
| Number of flowering branches | 29.28 | 10.52 | 99.40$^{**}$ | 17.90$^{*}$ | 5 | 26 | 7.3 | 21.7 |
| Number of non flowering branches | 25.56 | 38.30 | 310.15$^{*}$ | 13.55$^{ns}$ | 8 | 51 | 15.4 | 37.2 |
| Bottom leaves length | 16.29 | 10.23 | 273.46$^{**}$ | 5.27$^{ns}$ | 9.5 | 38 | 10.35 | 31.4 |
| Bottom leaves width | 23.96 | 4.01 | 63.15$^{**}$ | 3.18$^{ns}$ | 4 | 18 | 6.15 | 15.9 |
| Top leaves length | 20.47 | 4.14 | 29.94$^{**}$ | 5.29$^{ns}$ | 5 | 13 | 6.95 | 12.9 |
| Top leaves width | 25.58 | 1.19 | 6.81$^{**}$ | 1.11$^{ns}$ | 2 | 6 | 2.95 | 5.95 |
| Inflorescence length | 48.98 | 32.36 | 344.32$^{**}$ | 51.92$^{ns}$ | 2.9 | 50 | 5.56 | 29.37 |
| Inflorescence width | 48.81 | 25.72 | 132.60$^{**}$ | 11.94$^{ns}$ | 1.8 | 36.7 | 5.35 | 20.5 |
| Flower length | 11.72 | 0.06 | 1.08$^{**}$ | 0.35$^{ns}$ | 1.4 | 3.3 | 1.64 | 2.61 |
| Flower width | 11.91 | 0.05 | 0.98$^{**}$ | 0.06$^{ns}$ | 1.4 | 3.3 | 1.63 | 2.46 |
| Petal length | 22.43 | 1.11 | 8.04$^{**}$ | 0.085$^{ns}$ | 2 | 7 | 3.5 | 6.8 |
| Petal width | 33.98 | 0.2 | 0.35$^{**}$ | 0.14$^{ns}$ | 1 | 3 | 1 | 1.48 |
| Sepal length | 15.81 | 2.58 | 14.3$^{**}$ | 3.84$^{ns}$ | 5 | 15 | 8.3 | 12.5 |
| Sepal width | 17.98 | 0.81 | 4.25$^{**}$ | 0.63$^{ns}$ | 2 | 7 | 4 | 6.5 |
| Capsule length | 31.31 | 2.75 | 21$^{**}$ | 1.74$^{ns}$ | 2 | 10 | 1.3 | 8.1 |
| Capsule width | 31 | 0.93 | 7.11$^{**}$ | 0.79$^{ns}$ | 1.5 | 6 | 0.75 | 4.65 |

**Continue Table 2- Analysis of variances for the morphological traits of *H. perforatum* L. populations**

| Traits | Mean Square | | | | Range variation | | Means traits | |
|---|---|---|---|---|---|---|---|---|
| Source variation | Coefficient of Variation (CV)% | Error | Populations | Repeat | Min | Max | Min | Max |
| Dark glands density on the top leaves | 39.97 | 81.98 | 1938.65** | 80.62 ns | 1 | 89 | 3.1 | 59.1 |
| Dark glands density on the middle leaves | 28.65 | 138.75 | 4025.74** | 148.83ns | 6 | 120 | 14.9 | 99.9 |
| Dark glands density on the bottom leaves | 29.7 | 110.44 | 2848.75** | 54.76ns | 5 | 130 | 11.6 | 93.2 |
| Dark glands density on the petal | 20.79 | 16.36 | 156.15** | 18.77ns | 5 | 31 | 12.9 | 28.1 |
| Light glands density on the top leaves | 27.99 | 2279.16 | 90839.48** | 5711.88** | 17 | 417 | 49.8 | 390.9 |
| Light glands density on the middle leaves | 26 .23 | 4253.36 | 157798.2** | 4051.8ns | 25 | 643 | 49.6 | 443.2 |
| Light glands density on the bottom leaves | 34.28 | 3588.76 | 137279.47** | 3645.12ns | 2 | 667 | 18.8 | 497.7 |
| Fifth internodes' length | 85.28 | 0.8 | 5.24** | 0.38ns | 0.5 | 4.5 | 1.02 | 3.68 |
| Number of capsules | 84.7 | 509.59 | 7736.50** | 416.59ns | 0 | 198 | 1.7 | 119.2 |
| Number of flowers | 93.46 | 470.39 | 3720** | 652.4ns | 1 | 170 | 7.9 | 99.2 |
| Flower diameter before opening | 24.06 | 0.72 | 2.34** | 0.43 ns | 1 | 5.5 | 2.7 | 4.6 |
| Light glands density on the top leaves surface area | 32.37 | 937.87 | 24060.29** | 925.76ns | 2 | 302 | 28.4 | 179.9 |
| Light glands density on the middle leaves surface area | 45.04 | 1186.77 | 20513.88** | 924.11ns | 5 | 448 | 21.3 | 203.9 |
| Light glands density on the bottom leaves surface area | 55.27 | 594.31 | 1059.94** | 425.52ns | 0 | 241 | 5.2 | 122.2 |
| Dark glands density on the top leaves surface area | 42.37 | 14.23 | 299.95** | 5.12ns | 0 | 32 | 1 | 23.6 |
| Dark glands density on the middle leaves surface area | 48.21 | 10.29 | 202.7** | 13.46ns | 1 | 28 | 2 | 17.2 |
| Dark glands density on the bottom leaves surface area | 57.14 | 8.24 | 116.96** | 2.05ns | 0 | 24 | 2.1 | 15.7 |

ns, * and **: not significant, $p< 0.05$ and $p< 0.01$, respectively

The highest coefficients of variations were found in flowers number, the fifth internodes' length and capsule length (respectively, 93.46, 85.28 and 84.7%); however the lowest coefficients of variations were seen in flower width (11.72%) and sepal length (11.91 %). Therefore, the studied populations of St. John's wort showed remarkable variations in flowers and capsules numbers which are important traits in plant performances. The evaluation of St. John's wort populations revealed that the narrowest and widest leaves existed in Nahavand (with 3.96 leaf length: width ratio) and Ziyarat populations (with 1.65 leaf length: width ratio), respectively. Furthermore, the narrow-leaf populations had taller stems, larger leaves length, and greater dark and light glands density on the leaves

surface area as compared to the broad-leaf populations. However, the narrow-leaf populations had the lowest stem diameter, stem internodes number and leaves and inflorescences dimensions than those of others (Table 2).

In present study, the highest positive significant correlations were observed between flower length with flower width (0.98); dark glands density on the middle leaves with dark glands density on the bottom leaves surface area (0.95); petal length with flower length (0.9) and width (0.89); dark glands density on the middle leaves with dark glands on the top leaves (0.89); capsule length with capsule width (0.89); flowering branches number with branches number (0.88); light glands density on the middle leaves surface area with light glands density on the bottom leaves surface area (0.88). The lowest significant correlations were found between capsule length with flower length (0.013) and width (0.0001) (Table 3).

Evaluation the principal components of 39 traits in these populations indicated that four components explained 68.99% of total variance and the first three components had greater roles. The first principal component shared 30.42 % of the total variance and showed the positive coefficients for these traits such as main stem length; branches number; the largest and smallest secondary branches length; stem diameter; stem internodes number; flowering and non-flowering branches number; inflorescence length and width; dark and light glands density on the top and middle leaves; light glands density on the top, middle and bottom leaves; the fifth internodes' length; dark and light glands density on the top and bottom leaves surface area and flowers number. On the basis of this component, these traits were the most important parameters in the populations. Overall, the results indicated that leaves and flowers number, leaves dimensions and dark and light glands density on the leaves increased with rising stem height, flowering branches number and secondary branches number (Table 4).

The second principal component shared 19.91% of total variance and showed the positive coefficients for some traits such as the length and width of the bottom and top leaves, sepal, petal, flower, capsule length and width. Also the negative coefficients were observed for dark glands density on the petal and middle leaves surface area. The third principal component allocated 12.09% of total variance and its coefficients were positive for sepal width and flowers diameter and negative for capsule length and capsules number. The fourth principal component only showed a positive coefficient for the secondary stem length with 6.51% of total variance. In general, the principal component analysis results indicated that capsules, flowers, top and bottom leaves were the most important parts in evaluation of St. John's wort populations (Table 4).

The cluster analysis (using UPGMA clustering procedures) on the morphological characters arranged St. John's wort populations into four main groups. The first group had the highest stem height, branch number, stem diameter, the largest and smallest secondary stems length, the top and bottom leaves dimensions, inflorescence dimensions, and flowers and capsules number. Of course, the least dark glands density on the petals was observed in this group. The second group had the highest capsule dimensions, dark and light glands density on the leaves surface area and dark glands density on the petal. In general, the first and second groups had greater dark and light glands density on the leaves than the other groups (Figure 2).

**Table 3- The simple correlation (r) between values of *H. perforatum* L. populations for different botanical traits**

| Populations no. [a] | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | | | | | | | | | | | | | |
| 2 | .712** | 1 | | | | | | | | | | | | |
| 3 | .445* | .163 | 1 | | | | | | | | | | | |
| 4 | .598** | .481* | .656** | 1 | | | | | | | | | | |
| 5 | .743** | .482* | .222 | .298 | 1 | | | | | | | | | |
| 6 | .643** | .856** | .116 | .365 | .346 | 1 | | | | | | | | |
| 7 | .471* | .489* | .186 | .260 | .695** | .331 | 1 | | | | | | | |
| 8 | .547** | .882** | .089 | .412* | .157 | .803** | .036 | 1 | | | | | | |
| 9 | .742** | .443* | -.032 | .139 | .668** | .492* | .463* | .242 | 1 | | | | | |
| 10 | .604** | .191 | .087 | .165 | .633** | .212 | .343 | .017 | .777** | 1 | | | | |
| 11 | .471* | .037 | .044 | -.048 | .532** | .097 | .235 | -.109 | .717** | .683** | 1 | | | |
| 12 | .334- | .166 | .169 | .052 | .402* | -.084 | .153 | -.281 | .454* | .725** | .812** | 1 | | |
| 13 | .655** | .547** | .353 | .688** | .656** | .446* | .739** | .230 | .474* | .475* | .200 | .238 | 1 | |
| 14 | .476* | .546** | .364 | .550** | .376 | .492* | .569** | .340 | .334 | .284 | .113 | .073 | .627** | 1 |

**Continue Table 3- The simple correlation (r) between values of *H. perforatum* L. populations for different botanical traits**

| Populations no. [a] | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | .024 | -.016 | -.045 | .306 | -.005 | .019 | . -.272 | . .397* | .385 | .417* | .527** | -.273 | .381 | .609** | .673** | .750** |
| 2 | -.361 | -.394 | -.391 | -.011 | -.463* | -.263 | .164 | .120 | .314 | .439* | .481* | -.140 | .477* | .476* | .559** | .429* |
| 3 | .011 | -.014 | -.107 | -.022 | -.114 | .083 | -.031 | .032 | .271 | .146 | .130 | .013 | .294 | .312 | .419* | .253 |
| 4 | -.139 | -.158 | -.383 | -.070 | -.223 | -.020 | .166 | .223 | .127 | .123 | .324 | -.062 | .241 | .307 | .517** | .286 |
| 5 | .125 | .099 | .116 | .524** | .129 | .106 | .151 | .383 | .277 | .371 | .453* | .003 | .253 | .465* | .518** | .746** |
| 6 | -.295 | -.331 | -.330 | -.110 | -.350 | -.153 | .205 | .108 | .190 | .346 | .437* | -.293 | .444* | .417* | .488* | .198 |
| 7 | -.094 | -.089 | -.006 | .243 | -.115 | -.173 | -.036 | .164 | .330 | .505* | .552** | .054 | .402* | .371 | .305 | .516** |
| 8 | -.377 | -.416* | -.461* | -.164 | -.482* | -.210 | .236 | .079 | .168 | .229 | .257 | -.205 | .344 | .358 | .491* | .189 |
| 9 | .307 | .274 | .297 | .396* | .392 | .088 | .248 | .376 | .241 | .426* | .532** | -.336 | .335 | .567** | .462* | .641** |
| 10 | .548** | .503* | .432* | .372 | .563** | .246 | .145 | .339 | .163 | .216 | .311 | -.212 | .143 | .414* | .289 | .560** |
| 11 | .399 | .400* | .597** | .327 | .474* | .375 | .128 | .301 | .145 | .141 | .206 | -.296 | -.033 | .194 | .232 | .451* |
| 12 | .462* | .473* | .542** | .329 | .470* | .427* | .116 | .317 | .090 | -.004 | .121 | -.191 | -.210 | -.013 | -.008 | .325 |
| 13 | -.000 | .002 | -.150 | .260 | -.045 | -.038 | .169 | .364 | .245 | .403* | .602** | -.066 | .385 | .458* | .448* | .517** |
| 14 | -.048 | -.070 | -.324 | -.138 | -.108 | -.008 | .369 | .336 | .108 | .292 | .505* | .028 | .550** | .548** | .591** | .320 |
| 15 | 1 | .988** | .750** | .233 | .902** | .624** | .013 | .145 | -.300 | -.298 | -.206 | -.133 | -.241 | -.004 | -.021 | .095 |
| 16 | | 1 | .760** | .241 | .897** | .610** | .000 | .142 | -.277 | -.283 | -.175 | -.106 | -.264 | -.038 | -.065 | .077 |
| 17 | | | 1 | .236 | .682** | .562** | -.323 | -.160 | -.033 | -.137 | -.164 | -.111 | -.313 | -.214 | -.248 | .116 |
| 18 | | | | 1 | .217 | -.075 | .149 | .268 | .387 | .441* | .402* | .130 | .039 | .226 | -.008 | .650** |
| 19 | | | | | 1 | .551** | .022 | .201 | -.357 | -.309 | -.185 | -.245 | -.272 | .028 | .009 | .094 |
| 20 | | | | | | 1 | .080 | .185 | - | -.568** | -.312 | -.164 | -.393 | -.270 | .056 | -.064 |
| 21 | | | | | | | 1 | .899** | .549** | .012 | .179 | -.103 | .188 | .438* | .485* | .176 |
| 22 | | | | | | | | 1 | -.205 | -.045 | .170 | -.239 | .067 | .414* | .515** | .348 |
| 23 | | | | | | | | | -.234 | .893** | .702** | .387 | .612** | .453* | .012 | .541** |
| 24 | | | | | | | | | 1 | 1 | .864** | .341 | .788** | .658** | .186 | .558** |
| 25 | | | | | | | | | | | 1 | .244 | .712** | .648** | .316 | .622** |
| 26 | | | | | | | | | | | | 1 | .237 | -.011 | -.332 | .077 |
| 27 | | | | | | | | | | | | | 1 | .852** | .484* | .333 |
| 28 | | | | | | | | | | | | | | 1 | .756** | .545** |
| 29 | | | | | | | | | | | | | | | 1 | .400* |
| 30 | | | | | | | | | | | | | | | | 1 |
| 31 | | | | | | | | | | | | | | | | |
| 32 | | | | | | | | | | | | | | | | |
| 33 | | | | | | | | | | | | | | | | |
| 34 | | | | | | | | | | | | | | | | |
| 35 | | | | | | | | | | | | | | | | |
| 36 | | | | | | | | | | | | | | | | |
| 37 | | | | | | | | | | | | | | | | |
| 38 | | | | | | | | | | | | | | | | |
| 39 | | | | | | | | | | | | | | | | |

**Continue Table 3- The simple correlation (r) between values of *H. perforatum* L. populations for different botanical traits**

| Populations no. [a] | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | .451* | -.302 | -.304 | .261 | .235 | .386 | .085 | .058 | .116 |
| 2 | .375 | .383 | -.198 | .405* | .269 | .407* | .291 | .263 | .287 |
| 3 | .483* | -.032 | -.348 | .205 | .311 | .568** | .078 | .118 | .168 |
| 4 | .666** | .084 | -.628** | .166 | .248 | .526** | .046 | .089 | .233 |
| 5 | .465* | .565** | -.111 | .209 | .186 | .245 | .018 | .123 | .072 |
| 6 | .265 | .209 | -.259 | .448* | .172 | .282 | .219 | .114 | .168 |
| 7 | .356 | .731** | -.092 | .330 | .170 | .166 | .355 | .331 | .300 |
| 8 | .252 | .037 | -.182 | .303 | .235 | .380 | .159 | .138 | .177 |
| 9 | .146 | .290 | -.157 | .302 | .094 | .018 | .099 | -.132 | -.107 |
| 10 | .096 | .222 | -.190 | .115 | -.062 | -.113 | -.111 | -.275 | -.248 |
| 11 | .098 | .088 | -.167 | -.020 | -.050 | -.066 | -.154 | -.219 | -.255 |
| 12 | -.012 | .026 | -.227 | -.248 | -.267 | -.219 | -.248 | -.221 | -.215 |
| 13 | .595** | .515** | -.479* | .312 | .244 | .332 | .166 | .188 | .285 |
| 14 | .511** | .502* | -.468* | .296 | .314 | .417* | .137 | .165 | .241 |
| 15 | -.018 | -.164 | .017 | -.235 | -.303 | -.333 | -.350 | -.504* | -.495* |
| 16 | -.036 | -.163 | .010 | -.267 | -.306 | -.336 | -.306 | -.433* | -.430* |
| 17 | -.230 | -.263 | .268 | -.184 | -.346 | -.399* | -.127 | -.341 | -.377 |
| 18 | -.040 | .094 | .029 | -.008 | .117 | .007 | .152 | .275 | .262 |
| 19 | -.034 | -.087 | .060 | -.245 | -.282 | -.383 | -.372 | -.546** | -.558** |
| 20 | .221 | -.173 | -.031 | -.339 | -.408* | -.239 | -.605** | -.608** | -.568** |
| 21 | .333 | .235 | -.530** | .065 | .269 | .237 | -.291 | -.087 | -.043 |
| 22 | .449* | .357 | -.507** | -.037 | .180 | .139 | -.405* | -.180 | -.158 |
| 23 | -.249 | .045 | .084 | .476* | .480* | .423* | .774** | .719** | .742** |
| 24 | -.107 | .234 | -.036 | .686** | .605** | .474* | .836** | .727** | .749** |
| 25 | .063 | .279 | -.181 | .528** | .481* | .441* | .696** | .647** | .739** |
| 26 | -.198 | .066 | .096 | .215 | .159 | .146 | .381 | .514** | .537** |
| 27 | .105 | .251 | -.100 | .831** | .781** | .667** | .642** | .495* | .538** |
| 28 | .331 | .302 | -.242 | .692** | .776** | .669** | .392 | .306 | .314 |
| 29 | .735** | .296 | -.376 | .385 | .594** | .703** | -.005 | .032 | .004 |
| 30 | .208 | .348 | -.054 | .146 | .224 | .237 | .189 | .244 | .284 |
| 31 | 1 | .295 | -.568** | .159 | .322 | .523** | -.231 | -.060 | -.066 |
| 32 | | 1 | -.064 | .070 | .080 | .094 | .008 | .121 | .062 |
| 33 | | | 1 | -.188 | -.225 | -.330 | .144 | .076 | -.025 |
| 34 | | | | 1 | .727** | .569** | .612** | .370 | .379 |
| 35 | | | | | 1 | .881** | .501* | .536** | .502* |
| 36 | | | | | | 1 | .414* | .517** | .511** |
| 37 | | | | | | | 1 | .823** | .816** |
| 38 | | | | | | | | 1 | .956** |
| 39 | | | | | | | | | 1 |

* and **: $p< 0.05$ and $p< 0.01$, respectively

[a] 1-stem height; 2-number of branches; 3 and 4-respectively the smallest and largest secondary stems length; 5-stem diameter 6- number of stem internodes; 7 and 8- respectively numbers of flowering and non-flowering branches; 9 and 10-respectively the bottom leaves length and width; 11 and 12-respectively the top leaves length and width; 13 and 14- respectively inflorescence length and width; 15and 16- respectively flower length and width; 17 and 18- respectively petal length and width; 19 and 20- respectively sepal length and width; 21 and 22-respectively capsule length and width; 23, 24 and 25 -respectively dark glands density on the higher, middle and bottom leaves; 26- dark glands density on the petal; 27, 28 and 29- light glands density on the top, middle and bottom leaves; 30-the fifth internodes' length; 31 and 32- respectively numbers of capsules and flowers; 33-flowers diameter before opening; 34, 35 and 36 respectively light glands density on the top, middle and bottom leaves surface area; 37, 38 and 39- respectively dark glands density on the top, middle and bottom leaves surface area.

**Table 4 - Principal component analysis using morphological traits of *H. perforatum* L. populations**

| Traits | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| **Eigen value** | 11.866 | 7.767 | 4.717 | 2.559 |
| **Proportion of variance** | 30.426 | 19.915 | 12.094 | 6.561 |
| **Cumulative variance** | 30.426 | 50.341 | 62.435 | 68.996 |
| | **Eigenvector** | | | |
| | **PC1** | **PC2** | **PC3** | **PC4** |
| **Length stem** | 0.736 | 0.493 | -0.084 | -0.224 |
| **Number of branches** | 0.751 | 0.001 | -0.274 | -0.471 |
| **The smallest secondary stem length** | 0.396 | 0.107 | -0.187 | 0.425 |
| **The largest secondary stem length** | 0.559 | 0.131 | -0.449 | 0.143 |
| **Stem diameter** | 0.598 | 0.547 | 0.146 | -0.212 |
| **Number of stem internodes** | 0.626 | 0.053 | -0.307 | -0.454 |
| **Number of flowering branches** | 0.622 | 0.214 | 0.182 | -0.278 |
| **Number of non flowering branches** | 0.532 | -0.128 | -0.433 | -0.373 |
| **Bottom leaves length** | 0.501 | 0.675 | 0.226 | -0.268 |
| **Bottom leaves width** | 0.286 | 0.803 | 0.270 | -0.108 |
| **Top leaves length** | 0.141 | 0.739 | 0.293 | -0.077 |
| **Top leaves width** | -0.019 | 0.719 | 0.295 | -0.010 |
| **Inflorescence length** | 0.705 | 0.359 | -0.086 | -0.086 |
| **Inflorescence width** | 0.658 | 0.209 | -0.298 | 0.058 |
| **Flower length** | -0.343 | 0.738 | 0.250 | 0.310 |
| **Flower width** | -0.354 | 0.713 | 0.287 | 0.327 |
| **Petal length** | -0.393 | 0.566 | 0.549 | 0.082 |
| **Petal width** | 0.250 | 0.303 | 0.545 | 0.010 |
| **Sepal length** | -0.368 | 0.756 | 0.251 | 0.267 |
| **Sepal width** | -0.375 | 0.681 | -0.030 | 0.197 |
| **Capsule length** | 0.296 | 0.283 | -0.426 | 0.217 |
| **Capsule width** | 0.287 | 0.527 | -0.358 | 0.156 |
| **Dark glands density on the top leaves** | 0.625 | -0.271 | 0.621 | -0.008 |
| **Dark glands density on the middle leaves** | 0.787 | -0.207 | 0.542 | -0.002 |
| **Dark glands density on the bottom leaves** | 0.821 | -0.007 | 0.389 | -0.021 |
| **Dark glands density on the petal** | 0.099 | -0.424 | 0.396 | 0.274 |
| **Light glands density on the top leaves** | 0.802 | -0.194 | 0.150 | 0.236 |
| **Light glands density on the middle leaves** | 0.817 | 0.179 | 0.038 | 0.296 |
| **Light glands density on the bottom leaves** | 0.668 | 0.334 | -0.450 | 0.210 |
| **Fifth internodes' length** | 0.585 | 0.462 | 0.343 | -0.150 |
| **Number of capsules** | 0.431 | 0.317 | -0.606 | 0.239 |
| **Number of flowers** | 0.426 | 0.176 | -0.084 | -0.323 |
| **Flower diameter before opening** | -0.351 | -0.254 | 0.470 | -0.300 |
| **Light glands density on the top leaves surface area** | 0.659 | -0.205 | 0.119 | 0.237 |
| **Light glands density on the middle leaves surface area** | 0.686 | -0.261 | -0.003 | 0.522 |
| **Light glands density on the bottom leaves surface area** | 0.721 | -0.224 | -0.201 | 0.487 |
| **Dark glands density on the top leaves surface area** | 0.548 | -0.505 | 0.536 | 0.048 |
| **Dark glands density on the middle leaves surface area** | 0.560 | -0.572 | 0.392 | 0.094 |
| **Dark glands density on the bottom leaves surface area** | 0.599 | -0.557 | 0.361 | 0.108 |

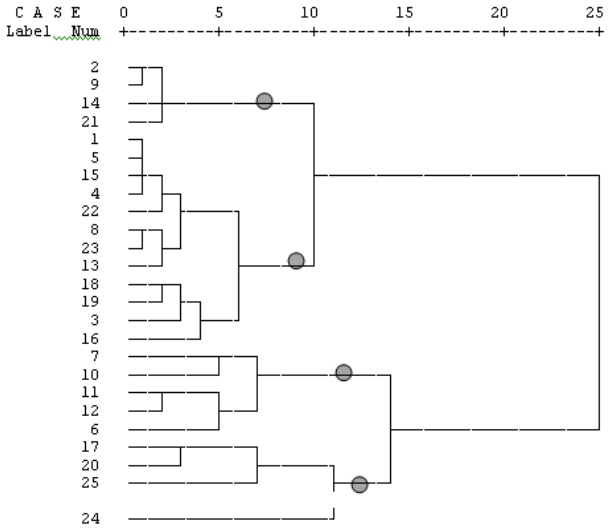


**Fig. 2- Dendrogram generated by cluster analysis of botanical traits (developed using UPGMA clustering procedures). The scales portray a dissimilarity index calculated using Euclidean distance coefficient**

The third group had the highest sepals, petals and flowers length and width, flowers diameter and stem internodes number. But the lowest branches number, dark glands density on the leaves, dark and light glands density on the leaves surface area were observed in the fourth group. On the basis of cluster analysis, we can select the best possible population for the breeding programs. Similarly, we found that the classification of the population according to morphological traits did not correspond to the geographical grouping of St. John's wort populations. In other words, the populations' morphology and their geographical distributions did not follow a similar pattern, as the populations from the same geographical origin entered into different clusters (e.g. five and six populations) and also the populations from different geographical origins entered the same cluster (e.g. 9 and 14 populations). Among the populations examined, those from Gilan, Mazandaran, Ardebil and Golestan populations were clustered in different groups, though they were from the regions with far geographical distances (Figure 2).

# Discussion

Based on our results, it was revealed that the populations of Alamut (85.56cm) and Chenaran (82.57cm) had the highest plant height and populations of Macrowave-west Rustam Abad (29.92cm) and Sisakht (29.99cm) had the lowest plant height. It found that the largest top and bottom leaves dimensions of Alamut population were measured 15.9 to 34.4 mm for leaves length and 5.9 to 15.9 mm for leaves width. The smallest top and bottom leaves dimensions belonged to Plo-e Sefid population with the leaves length of 7.4 to 10.35 mm and leaves width of 3.9 to 5.25 mm). The largest and smallest dimensions of flowers, sepals and petals were related to the population which gathered from Zanjan/Tarum and Noor cities, respectively. It found that different climate conditions affected on leaves, flowers, sepals and petals dimensions and plant height (Table 2).

Hosseini and Dori reported that the plant height of St. John's wort populations from Garmadasht (120.3cm) was almost two times higher than that of Derazno population

(67.7cm). They stated that the shorter plant height of Derazno region might be due to genetic origin differences or plant acclimatization of St. John's wort with regard to the high altitude of Derazno region. In both the regions no significant correlation between the flowering branches and plant height were stated [11].

Roblek et al. studied *H. perforatum* L. morphological characteristics in different altitudes. They found that plant height decreased at high altitudes due to reduction of stem internodes number and also decrease of leaf chlorophyll content. They found that flowers number decreased as altitude increased (147 flowers at 400 m a.s.l. and 50 flowers at 1700 meters above sea level) [18].

The results of a research conducted in Lithuania (Bagdonaite et al.) on genotypes origin of St. John's wort (18 populations and 2 reformed species) indicated that these populations differed in their plant height, sepal length, raw weight, inflorescence dimensions, leaves traits, dark glands density on the leaves, but the inflorescence length did not significantly differ among the genotypes. The width of inflorescence varied from 10.9 to 15.5 cm. Overall, the results indicated that Lithuanians' genotypes of *H. perforatum* L. were superior to improved varieties in respect of plant height, inflorescence length and width and leaves length and width [1]. Some research conducted on *H. perforatum* L. populations revealed that different populations varied in their plant height, stem internodes number, leaves and petals dimensions and other traits [4, 19, 21].

Our study showed that this comparison of leaves dimensions was an indication for morphological evaluations of St. John's wort populations and climatic conditions impacted on the plant growth in these regions. It found that the density of dark glands varied on the top leaves from 3 to 59 glands, on the middle of leaves from 15 to 100 and on the bottom leaves from 1 to 130 glands (Table 2). In this study, the result indicated that dark and light glands density on the top and bottom leaves enhanced in company with increasing dark and light glands density on the middle leaves. In other word, dark and light glands density had correspondingly varied on the different leaves of this plant (Table 3).

Donald et al. studied the effects of light on morphological characteristic and dark glands density on St. John's wort leaves and stated that the light and other environmental parameters had a major consequence on these characteristics. The increasing light intensity augmented stems biomass and development of branches; enhanced the photosynthesis capacity and carbon fixation; and finally dark glands number [5]. Our results are also in agreement with the results of Donald et al. [5].

Based on results, it was revealed that morphological variants of leaves in *H. perforatum* are the narrow-, intermediate-, and broad-leaf varieties [3, 20]. A leaf length: width ratio of 3.1 and 2.0, and a light gland density of 5.7 and 1.7 glands/mm2 leaves characterize the narrow- and broad-leaf varieties, respectively [20, 23]. Southwell and Campbell studied *H. perforatum* L. populations in Australia, reported that the narrow-leaf populations contained more dark glands (6.2 glands per $mm^2$) than the broad-leaf populations (2.1 glands per $mm^2$) [20]. Our results are also in agreement with the results of Southwell and Campbell [20].

Walker et al. studied the morphological characteristics of St. John's wort populations in California, Montana and Oregon. Their results showed that California population had larger leaf length/ width ratio, greater dark glands density on the leaves and taller stems than those from Montana population. In

addition to the leaf length/width ratio, differences were detected in the following morphological characteristics: leaf dark gland density, leaf light gland density, leaf area, and primary stem length [23]. Our results are also in agreement with the results of Walker et al. [23].

Based on our research results, it was revealed that significant and negative correlations were observed among sepal and petal length with non-flowering branches number, capsule length and width with flower diameter. While significant and positive correlations were observed among capsule width with capsules number, sepal width with the fifth internodes' length (Table 3).

Naghdi Badi et al. reported that sepal length with inflorescence width had a positive significant correlation (0.979), but a negative significant correlation was observed between sepal length with non-flowering branches number (-0.924). Moreover they found significant and negative correlation between sepal width with dark glands density on the petal (-0.899), and a positive significant correlation between it with non-flowering branches (0.924). They reported significant and positive correlations between light glands density with leaf length: width ratio (0.936) and petal length (0.937) [14]. Our results are also in agreement with the results of Naghdi Badi et al. [14]. Bagdonaite et al. in a study on St. John's wort genotypes in Lithuanian realized that there were the highest significant correlations among inflorescence, leaves, and petals length and width [2].

Our results indicated that leaves and flowers number, leaves dimensions and dark and light glands density on the leaves increased with rising stem height, flowering branches number and secondary branches number. Based on results of our study showed that every environmental condition (location) had an enormous effect on the growth and development, plant reproduction, height, leaves, flowers and inflorescence dimensions, etc. of *H. perforatum* L. populations (Table 2 and 3).

Lebaschi et al. studied four St. John's wort populations in natural habitats in Iran and reported that among the natural habitats of Gorgan, Noshaher, Siahkal and Khalkhal, the highest plant performance was related to Gorgan and Siahkal populations [12]. Fox et al. studied the effect of summer precipitation and winter temperatures on growth and reproduction of *H. perforatum* L. Their results showed that both summer rainfall regimes and winter warming modified the plant performance, in a way that the winter warming had positive effects with earlier initiation of plant growth but it had strong negative effects on plant height, inflorescence and flowering and reproduction. Summer drought reduced reproductive yield. It found that plants modify acquisition of carbon and nitrogen, leaf water content, surface temperature, and secondary compounds in drought conditions. But summer drought did no direct impact on plant height and flowering [8].

Ejtehadi et al. investigated St. John's wort populations in some parts of Khorasn province of Iran and reported that topography and degree and point of slope affected the dispersal of plants more than above sea level factor [7]. Dori et al. gathered St. John's wort populations from different altitudes of Golestan province and reported that each habitat has got its own range of suitable conditions for the plant growth [6]. Bagdonaitė1 et al. determined that the vast ecological adaptation of St. John's wort is depended on the kind of population [2]. The present results are also in agreement with the results of Bagdonaitė1 et al. [2].

## Conclusion

Our results showed that St. John's wort is a perennial species with variability in morphological characteristics. Moreover, the clustering of traits and parameters, the strong correlations of traits and large morphological variations existed among the St. John's wort populations in different natural habitats of Iran indicated that these populations are important genetic resources and initial breeding material which could be utilized in future breeding programs.

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