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Evaluation of Some Phytochemical Characteristics of Henna (*Lawsonia inermis* L.) Populations under Jiroft Climate Condition

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ABSTRACT

Lawsonia inermis L. (Henna), which belongs to the Lythraceae family, is widely recognized for its use in traditional medicine, cosmetics, and textile dyeing, primarily due to its rich bioactive compounds. This study aimed to evaluate the total phenolic content, total flavonoid content and antioxidant properties of methanolic extracts from 14 different Iranian populations of *L. inermis* cultivated in a completely randomized block design (RCBD) with three replicates in Jiroft, Kerman province. The phenolic and flavonoid contents were quantified using Folin-Ciocalteu and aluminum chloride colorimetric assays, respectively, while the DPPH radical scavenging assay was conducted using a concentration range of 0.1 mmol/l DPPH in methanol. Calibration curves were prepared using a series of standard solutions of known antioxidant compounds, such as ascorbic acid, to determine the IC50 values of the henna extracts. The results demonstrated significant genotypic variations in all measured traits. The populations from Lamerd, BandarLenge, Borazjan 2, GhaleGhazi 1, and Ghaleganj exhibited the highest total phenolic contents, whereas GhaleGanj had the highest flavonoid content. The Lamerd population displayed the highest antioxidant activity with the lowest IC50 value. Cluster analysis grouped the populations based on their biochemical traits, reflecting the genetic and ecological influences on their phytochemical profiles. These findings highlight the potential for selecting superior henna populations to maximize the production of bioactive compounds, thereby enhancing their application in pharmaceutical and cosmetic industries. This study provides a scientific foundation for optimizing henna cultivation and utilization strategies to improve product quality and therapeutic efficacy.

Keywords: Bioactive compounds, DPPH assay, Genetic variability, Pharmaceutical applications, Phytochemical analysis

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INTRODUCTION

Lawsonia inermis L. (Henna), which belongs to the Lythraceae family, is a perennial plant widely recognized for its applications in traditional medicine, cosmetics, and textile dyeing due to its bioactive compounds [1,2]. The natural components of *L. inermis* include lawsone (2-hydroxy-1,4-naphthoquinone), essential oil, tannins, terpenoids, lipids, coumarins, and a variety of flavonoids such as linarigenin (4'-methoxyapigenin), apigenin 7-glucoside, apigenin-glucoside, luteolin, luteolin 7-glucoside, and cosmosiin. Additionally, phenolic acids like gallic acid and *p*-coumaric acid are also present [3]. Predominantly cultivated in tropical and subtropical regions, henna has gained significant attention for its pharmacological properties, including antimicrobial, anti-inflammatory, and antioxidant activities. These therapeutic effects are primarily attributed to the plant's rich content of phenolic compounds and flavonoids [4,5]. Phenolic compounds and flavonoids are secondary metabolites in plants that play vital roles in defense against pathogens and oxidative stress. These compounds are also important for human health due to their antioxidant properties, which help in neutralizing free radicals and reducing the risk of chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders [6]. *L. inermis*, known for its vibrant dye, Lawson, is also rich in other phenolic compounds and flavonoids, making it an important subject for phytochemical research [7, 8]. Previous investigations on Iranian *L. inermis* aqueous leaf extracts revealed diverse antioxidant activities associated with ecotypes [9]. In another study, the Tunisian *L. inermis* study demonstrated that the butanolic fraction of the leaves exhibits potent antioxidant properties. These activities were primarily attributed to the presence of phenolic glycosides, including 1, 2, 4-trihydroxynaphthalene-1-O-β-D-glucopyranoside [10]. Previous studies have shown significant genetic variability in *L. inermis*, influencing the biosynthesis of phenolic and flavonoid compounds. For

instance, research has indicated that different genotypes can vary widely in their total phenolic content and antioxidant activities, which are crucial for their medicinal and cosmetic properties. This variability can be influenced by several factors including environmental conditions, soil type, and agricultural practices [9, 11]. This genetic diversity underpins the importance of selecting optimal populations for cultivation. Iran, with its diverse climatic conditions, provides a unique opportunity to study the variations in phytochemical constituents of henna plants from different regions [12]. Despite the traditional use of henna in Iranian culture, comprehensive studies evaluating the phenolic and flavonoid content, as well as the antioxidant properties of henna from different Iranian populations, are limited. Understanding these variations is crucial for optimizing the use of *L. inermis* in pharmaceutical and cosmetic industries. Jiroft, located in the southeastern part of Iran, offers a distinctive environment for cultivating henna due to its favorable climatic conditions [13]. By cultivating henna populations from different regions of Iran in Jiroft, this study aims to minimize environmental variability and focus on the intrinsic differences among the populations. Jiroft's climatic conditions, characterized by high solar exposure, elevated temperatures, and arid conditions, provide an ideal environment for cultivating henna. These conditions can enhance the synthesis of phenolic compounds and flavonoids, contributing to the higher bioactive compound content observed in the studied populations. By minimizing environmental variability, this study focused on intrinsic genetic differences, providing a clearer understanding of the genotypic influences on phytochemical profiles. This study aims to evaluate the total phenolic and flavonoid content, along with the antioxidant properties, of extracts from 14 different populations of henna collected from various parts of Iran and cultivated in Jiroft, Kerman province. By assessing these parameters, we aim to identify the populations with the highest bioactive compound content and antioxidant potential, thereby providing a scientific basis for the selection of superior henna populations for cultivation and industrial applications.

MATERIALS AND METHODS

Plant Material and Growing Conditions

Fourteen populations of *L. inermis* were collected from four provinces (Table 1). These populations were cultivated at the Farm of the Center for Research and Education of Agriculture and Natural Resources in southern Kerman province, Jiroft, in 2020 (latitude 28° 28′ N, longitude 57° 50′ E, at an altitude of 597 meters). The fourteen populations were propagated via seeds and planted with spacing of 50 × 60 cm in a completely randomized block design (RCBD) with three replicates. The mean annual temperature of the region was 21.5 °C, and the mean annual precipitation was 220 mm. Leaves from three-year-old plants were harvested at the full flowering stage in late June 2023 for phytochemical analysis. The leaves were washed with water to remove dirt and then air-dried in the Medicinal Plants laboratory at the University of Hormozgan. The dried leaves were stored in a dry place until further use.

Preparation of Methanol Extract

To ensure consistency in the methanol extraction process, all samples were processed under identical conditions. The samples were ground to a fine powder. The dried leaf powder from each population was subjected to the same solvent-to-sample ratio, extraction time, and temperature conditions. Fifty grams of the dry powder from each sample were placed into one-liter volumetric flasks, and 200 mL of methanol was added to each flask. The mixtures were agitated on a shaker for 48 hours at room temperature, followed by filtration through filter paper. The resulting extracts were concentrated using a rotary evaporator under reduced pressure at 45 °C to remove the solvent. The final residues were further dried by incubation at 50 °C. Ten milligrams of each dried extract were then dissolved in 1 mL of distilled water to prepare a dilute extract.

Total Phenolic Determination

The total phenolic content in the methanol extracts was quantified using the Folin-Ciocalteu method [14]. Approximately 500 µL of the diluted extract was combined with 2 mL of Folin-Ciocalteu reagent.

After a 5-minute incubation in the dark, 2.5 mL of 7.5% sodium carbonate solution was added. The mixture was then kept in the dark at room temperature for 120 minutes. The absorbance was measured at 760 nm. The total phenolic content was expressed as micrograms of gallic acid equivalents per milligram of extract (µg GAE/mg Extract). Data for triplicate analysis were presented as mean ± SD.

Total Flavonoid Determination

The total flavonoid content of the extracts was determined using the aluminum chloride colorimetric assay [15]. To a volumetric flask containing 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water, 500 µL of the diluted extract was added. After a 30-minute reaction at room temperature, the absorbance of the solution was measured at 415 nm. The total flavonoid content was expressed as micrograms of quercetin equivalent per milligram of extract (µg QE/mg Extract). Data were presented as mean ± SD.

Determination of free Radical-scavenging Capacity (DPPH assay)

The antioxidant activity of the methanolic extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay [16].

Table 1 Characteristics of different *L. inermis* L. populations cultivated in Jiroft

No.	Population name	Province	Altitude (m)	Longitude (E)	Latitude (N)
1	Jiroft 1	Kerman	770	57º 44′	28º 43′
2	Jiroft 2	Kerman	616	57º 50′	28º 32′
3	Jiroft 3	Kerman	650	57º 46′	28º 34′
4	Zehkalout 1	Kerman	386	58º 35′	27º 47′
5	Zehkalout 2	Kerman	377	58º 38′	27º 44′
6	Zehkalout 3	Kerman	416	58º 31′	27º 49′
7	Borazjan 1	Bushehr	68	51º 13′	29º 16′
8	Borazjan 2	Bushehr	86	51º 11′	29º 13′
9	Borazjan 3	Bushehr	149	51º 14′	29º 17′
10	Lamerd	Fars	417	53º 11′	27º 20′
11	GhaleGhazi 1	Hormozgan	47	56º 32′	27º 26′
12	GhaleGhazi 2	Hormozgan	43	56º 33′	27º 27′
13	BandarLenge	Hormozgan	34	54º 52′	26º 33′
14	GhaleGanj	Kerman	405	57º 52′	27º 31′

To 1 mL of the diluted extract, 1 mL of a methanolic solution containing 0.1 mmol/L DPPH was added and the mixture was incubated in the dark for 15 minutes. The absorbance was then measured at 517 nm. The percentage inhibition of the DPPH radical was calculated using the following equation:

$I (\%)= (A_0-A_s/A_0)\times100$

Where A_0 is absorbance of control and A_s is the absorbance of tested sample. The IC_{50} values were calculated as the concentration of extract that could scavenge 50% of DPPH radical.

Statistical Analyses

The data represent the mean values of triplicate measurements, with results expressed as means ± standard deviation (SD). Statistical analysis was conducted using SAS software (version 9.1.3). A one-way analysis of variance (ANOVA) was performed, and significant differences between group means were identified using Duncan's multiple range test, with a significance threshold set at $p< 0.05$. Cluster analysis was conducted using PAST statistical software, employing the Ward method and Euclidean distances for the construction of the dendrogram.

RESULTA AND DISCUSSION

Given the influence of genotype on the phytochemical characteristics of medicinal plants, this study aimed to assess the quality, bioactive compounds, and antioxidant capacity of 14 henna populations cultivated in the Jiroft region over a growing season to improve product quality. The results demonstrated that genotype had a significant effect on all the traits studied (Table 2). The analysis of variance (ANOVA) for phytochemical attributes is presented in Table 2. Henna genetic resources (populations) significantly influenced total flavonoid content, free radical-scavenging capacity (DPPH) ($p < 0.05$), and total phenolic content ($p< 0.01$).

Total Phenolic Contents

The total phenolic contents of the methanolic extracts are presented in Table 3. The highest total phenolic content was observed in the populations from Lamerd, BandarLenge, Borazjan 2, GhaleGhazi 1, and Ghaleganj, with values of 127.16, 118.94, 117.89, 112.16, and 111.83 µg gallic acid/g extract, respectively. The populations with the lowest total phenolic content (µg GAE/g) were Zehkalout 1 (93.77), Jiroft 1 (96.05), Jiroft 2 (98.00), Zehkalout 2 (98.05), Jiroft 3 (98.16), and Zehkalout 3 (98.66) (Fig. 1).

Our findings align with previous studies that reported a total phenolic content of 96.76 µg gallic acid per g [17]. Another study on *L. inermis* revealed significant differences in total phenolic content between the genotypes 'Kahnooj' (165.03 µg gallic acid/g DW) and 'GaleGanj' (22.08 µg gallic acid/g DW) [18]. Therefore, extensive genetic variation among populations may contribute to differing physiological interactions that influence the production and accumulation of phenolic compounds.

Total Flavonoid Contents

The total flavonoid content yields of different populations are presented in Table 3. Significant differences were observed among populations in terms of total flavonoid content. The extract yields ranged from 43.33 to 123.33 micrograms of quercetin equivalent per gram of extract (Fig. 2). The highest yield was obtained from the GhaleGanj population (123.33 µg QE/g), while the Jiroft 3 population exhibited the lowest yield (43.33 µg QE/g).

The total flavonoid contents in the leaves of all populations were higher than those previously reported by Goudjil *et al*. [19] from Algeria and El Massoudi *et al*. [20] from Morocco. Hosseiny *et al*. (2023) documented that the total flavonoid contents of *L. inermis* ranged from 13.08 to 98.04 µg catechin/g dry matter [18]. The observed differences in phenolic and flavonoid contents among the different populations could be attributed to genetic variations that influence the biosynthesis of these compounds.

Antioxidant Activities

The antioxidant activities were measured using the DPPH assay and represented as IC50 values (µg/ml), which indicate the concentration of plant extract required to reduce the initial concentration of DPPH radicals by fifty percent. Thus, the highest antioxidant activity corresponds to the lowest IC_{50} value of the extract.

Table 2 Analysis of variance of the measured traits in different studied *L. inermis* L. populations

S.O.V.	df	Mean of squares		
		Total phenolic content	Total flavonoid content	Antioxidant activity
Replication	2	47.02 ^{ns}	45.24 ^{ns}	306.74 ^{ns}
Populations	13	310.86 **	1285.71*	73725 *
Error	26	80.62 ^{ns}	593.96 ^{ns}	178.77 ^{ns}
CV (%)	-	8.47	23.89	14.22

*, **, ns: significant at 5% and 1% probability levels and non-significant, respectively

Table 3 Mean comparison of total phenolic content, total flavonoid content and antioxidant activity in the studied populations of *L. inermis*

No	Population name	Total phenolic content (µg GAE/g Extract)	Total flavonoid content (µg QE/g Extract)	DDPH IC50 (µg/ml)
1	Jiroft 1	96.05 de ± 4.16	54.75 bc ± 3.35	674.52 c ± 3.18
2	Jiroft 2	98.00 de ± 5.22	56.67 bc ± 3.27	662.76 c ± 2.01
3	Jiroft 3	98.16 de ± 5.12	43.33 c ± 1.12	680.64 c ± 3.58
4	Zehkalout 1	93.77 e ± 3.59	66.67 bc ± 2.05	798.44 e ± 2.61
5	Zehkalout 2	98.05 de ± 4.12	58.67 bc ± 2.17	672.66 c ± 5.01
6	Zehkalout 3	98.66 de ± 5.17	73.33 bc ± 2.24	756.34 d ± 4.66
7	Borazjan 1	101.05 cde ± 7.85	83.33 abc ± 3.26	582.84 b ± 2.74
8	Borazjan 2	117.89 abc ± 6.52	66.67 bc ± 2.65	592.56 b ± 7.34
9	Borazjan 3	104.61 bcde ± 7.32	93.33 ab ± 2.45	586.42 b ± 6.84
10	Lamerd	127.16 a ± 6.62	53.33 bc ± 1.06	478.58 a ± 3.28
11	GhaleGhazi 1	112.16 abcd ± 6.21	63.33 bc ± 2.32	738.82 d ± 1.30
12	GhaleGhazi 2	107.05 bcde ± 8.32	83.33 abc ± 2.76	718.38 cd ± 2.79
13	BandarLenge	118.94 ab ± 7.82	86.67 abc ± 2.72	752.26 d ± 7.34
14	GhaleGanj	111.83 abcd ± 5.43	123.33 a ± 3.62	516.37 ab ± 1.66

Values in the same row followed by the same letter are not statistically different in Duncan's multiple range test (P < 0.05).

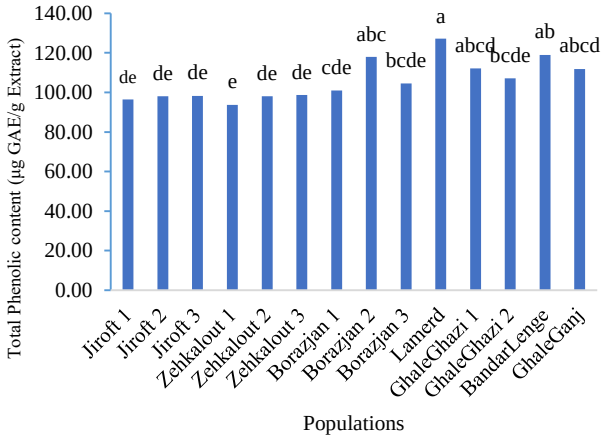

Fig. 1 Total phenolic contents of extract in the studied populations of *L. inermis*

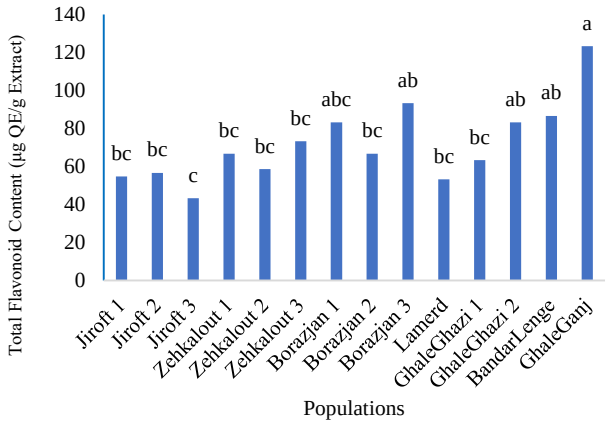

Fig. 2 Total flavonoid contents of extract in the studied populations of *L. inermis*

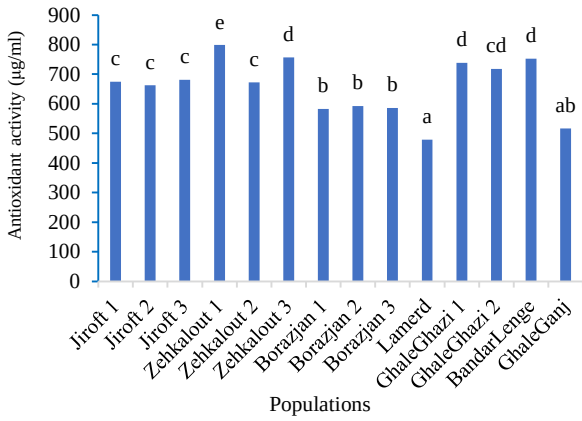

Fig. 3 Antioxidant activity of extract in the studied populations of *L. inermis*

The mean comparison results of antioxidant activity revealed that Lamerd exhibited the highest antioxidant activity, with the lowest IC50 value of 478.58 µg/ml. Conversely, Zehkalout 1 demonstrated the lowest antioxidant activity, with the highest IC50 value of 798.44 µg/ml (Table 3, Fig. 3). The high antioxidant potency of *L. inermis* leaves is likely attributed to their rich phenolic and flavonoid contents [21, 22]. Numerous previous studies have reported the high antioxidant activity of *L. inermis* [23]. For instance, Hasan *et al*. (2016) documented a high DPPH radical scavenging activity of *L. inermis*, measuring 79.16 ± 0.98 % [24]. In another study, the radical scavenging activity of the extracts was evaluated using the DPPH assay, with the highest activity observed in *L. inermis* extract (IC50 = 671.6 µg/ml) [17].

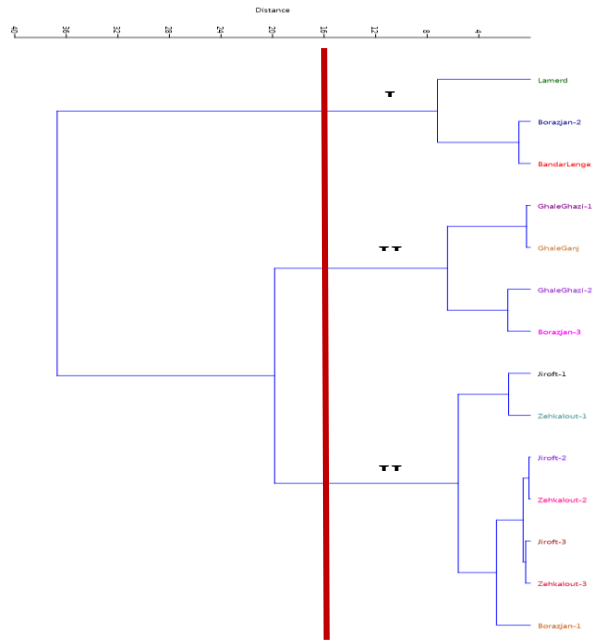

Fig. 4 Dendrogram of cluster analysis for the studied populations of *L. inermis* based on biochemical traits

Ecological factors, such as soil composition, microclimate, and altitude of the original growing locations, may also play a role. For example, populations from Lamerd and BandarLenge might have genetic traits that enhance their ability to produce higher levels of bioactive compounds under specific environmental conditions.

Cluster Analysis

The cluster analysis was performed based on all the examined parameters (Fig. 4) to calculate the approximate relationship among the used treatments. The first group included the populations of Lamerd, Borazjan 2, and BandarLenge, characterized by high total phenolic and flavonoid contents.

The second group comprised the populations of GhaleGhazi 1, GhaleGanj, GhaleGhazi 2, and Borazjan 3, which exhibited moderate total phenolic and flavonoid contents. The third group consisted of the remaining seven populations, which had low total phenolic content and low antioxidant activity. According to previous reports, the ecological and genetic backgrounds likely influence the biosynthesis of phenolic compounds, contributing to the biochemical polymorphism observed among populations [25]. Our results also indicated that higher antioxidant activity levels in these populations were correlated with increased total phenol and flavonoid contents. It has been well established that medicinal plants rich in phenolic and flavonoid contents exhibit high antioxidant activities [26].

CONCLUSION

The study evaluated the antioxidant properties, total phenolic content, and total flavonoid content of *L. inermis* L. extracts from 14 different Iranian populations cultivated in Jiroft. The results demonstrated significant genotypic differences in these phytochemical traits, with some populations showing higher levels of bioactive compounds and antioxidant activities than others. To maximize phenolic and flavonoid contents, it is recommended to select henna populations with genetically higher levels of these compounds, such as those from Lamerd, BandarLenge, and GhaleGanj. Cultivating these populations in regions with high solar exposure, elevated temperatures, and arid conditions similar to Jiroft could further enhance their bioactive compound production. Additionally, implementing consistent agricultural practices, such as proper spacing, regular irrigation, and organic fertilization, can help maintain high phytochemical yields. The antioxidant activity, measured using the DPPH assay, was also highest in the Lamerd population. These findings underscore the genetic variability among the different henna populations, suggesting that certain genotypes are more potent sources of antioxidants. The superior henna populations identified in this study, particularly those with high phenolic and flavonoid contents and strong antioxidant activities, have significant potential for pharmaceutical and cosmetic applications. For example, these populations can be used to develop natural antioxidant supplements, anti-inflammatory creams, and skin care products that capitalize on the high bioactive compound content. Additionally, the potent antioxidant properties of these henna extracts could be utilized in the formulation of hair dyes and conditioners that provide both coloring and protective benefits. This study highlights the importance of selecting optimal henna populations for cultivation to maximize the production of beneficial bioactive compounds. The cluster analysis further supported the differentiation among populations based on their biochemical traits, suggesting a strong influence of genetic and possibly ecological factors on the phytochemical composition of henna. Therefore, the findings provide a scientific basis for targeted breeding and cultivation strategies to enhance the use of henna in various industries, ultimately contributing to improved product quality and efficacy.

Conflict of interest

The authors declare that there is no conflict of interest.

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