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Malvaceae spp. leaves as a novel crop for food

Eyal Ben-Simchon¹, Eliezer Sapir ^{1,2}, Yiftach Vaknin¹, Oren Shelef^{1*}

¹Department of Natural Resources, Institute of Plant Sciences, Agricultural Research Organization, Rishon LeZion, Israel ²The Hebrew Univ. of Jerusalem, R.H. Smith Faculty of Agriculture, Food & Environment, Rehovot, Israel

*Corresponding author email: shelef@volcani.agri.gov.il

Abstract

Local wild plants are often used as a complementary food source for indigenous societies around the globe. However, domestication of such plants as new crops for food is facing serious challenges, so in fact only a handful of new crops were domesticated for commercial use in the last decades. Several *Malva* and *Lavatera* species (Family: Malvaceae) are ruderal, nitrophilic plants that very commonly dominate vegetation of field margins in Mediterranean agroecosystems. In Israel, those Malvaceae species - mainly *M. nicaeensis* All. and *L. cretica* L. - are commonly called "*Hubeza*" (Arabic: bread). *Hubeza* have an important role in the local cuisine. Traditionally, people are harvesting and eating the green leaves fresh, boiled or fried. The goal of this project is to test the potential of *M. nicaeensis* and *L.cretica* as a potential crop for human food. We hypothesize that the nutritional values of the *Hubeza* leaves is competitive to spinach, as well as other green leaves which are available commercially, and used in similar fashion. To test our hypothesis we compared nutritional values of wild *Hubeza* leaves to the nutritional values of other green leaves crops, in terms of, total N, P, K, Na, Fe, and Cu. We found that leaves of wild Malvaceae exhibited similar concentrations of nutrients as compared to commercial green leaves, cultivated in the field. The use of native plants for agriculture can increase biodiversity of agroecosystems, promote diversification of agriculture, and reduce agricultural inputs. The value of native plants, local production, and maintenance of agroecosystem biodiversity can reduce food millage, promote food diversity and agricultural resilience.

Key words: green leaves, Malva nicaeensis, Lavatera cretica, nutritional value, traditional food, novel crops.

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Introduction

Sustainable agriculture is the economically viable production of food, that is nonexploitative, socially responsible, and which serves as the foundation for future generations (Allen, Van Dusen, Lundy, & Gliessman, 1991). Shelef et al. (2018) illustrated how the use of local food production is reflecting the challenges and potential of developing more sustainable practices in agriculture. Using local plants is likely to prefer varieties that were naturally selected to tolerate the local environment, thus saving inputs, food millage, and empower regenerative agriculture (O. Shelef, Weisberg, & Provenza, 2017). According to the most serious attempt to estimate the value of wild edible plants, we are familiar with a range of about 30,000 edible plants globally (Food Plant Solutions, Bruce French), out of which we are commercialising merely 150 as crops (Sethi, 2015). Diversifying local production will help to foster more sustainable agroecosystems, create more efficient supply chains, and ultimately increase food security. The Mediterranean area was the cradle to some of the most important agriculture crops and practices (Gupta, 2004). Hundreds of Mediterranean plant species are classified as Crop Wild Relatives (Barazani et al., 2017), and many others are considered as protein rich

plants, e.g. oaks (Ozcan, 2006), and legumes (Barazani, Perevolotsky, & Hadas, 2008) such as *Lathyrus aphaca, Lotus edulis*, and *Pisum fulvum* (Dafni, 1985; Krispil, 1983–1989; Mayer-Chissick & Lev, 2014). Additionally, many of the wild annual greens that grow wild during the rainy season are edible and have a cultural history of being used as food and medicine (Mayer-Chissick & Lev, 2014). This suggests that the study of local plant crops, specifically in Israel and in the Mediterranean region, has great potential to support sustainable agriculture.

The Malvaceae family includes over 4000 species many of which are of economical and agricultural importance such as cotton (*Gossypium spp.*), kenaf (*Hibiscus cannabinus*), cacao (*Theobroma cacao*) and Okra (*Abelmoschus esculentus*). Of these, 32 species are found wild in Israel including several edible species.

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The generic term for these edible plants, "Hubeza," comes from the Arabic word "Hubez," meaning bread. It is not surprising that "Halamit" (Malva spp.) and "Maog" (Lavatera spp.) have a rich history in local culinary culture. Perhaps the earliest mention dates back to the third century in the Jewish oral law, the Mishnah (Mishnah Kil'ayim 1:8). Malvaceae paviflora (one of the wild species in Israel) exhibits antiinflammatory and antioxidant activity (Bouriche, Meziti, Senator, & Arnhold, 2011). However, there is little research documenting the nutritional value M. paviflora and related Hubeza. Barros et al. (2010) examined the nutritional and pharmaceutical value of M. sylvestris, also found in Israel. Their findings supported reports on the benefits of M. sylvestris as a nutritious additive to the diet (Guarrera, 2003), using fresh or boiled leaves, and green fruits (Neves, Matos, Moutinho, Queiroz, & Gomes, 2009) without any apparent adverse effects, even when high levels of nitrates were a concern (Hanningtonkiff, 1984).

This study evaluates several nutritional aspects of *Hubeza spp*. focusing on minerals: N, P, K, Fe, Cu and Na. This was accomplished through the sampling, drying and analysing wild leaves of individual plants in several populations, and comparing it to commercially-grown green leaves. We seek to confirm if *Hubeza spp*. holds nutritional significance for it to be foraged commercially or grown as a food crop.

Materials and Methods

Sample collection. We collected fresh Hubeza leaves (M. nicaeensis and L.cretica), at January -February 2019. For clarification - at this stage we could not differentiate between the two species, as the main distinguishable difference is the characteristics of the flowers and microscopic morphology of the leaf epidermis. A total of 51 Hubeza individuals were sampled from three locations in Israel: Moshav Bnei Re'em (31°46'N 34°47'E), Kibbutz Hagoen (32°21'N 34°55'E), and the Volcani Institute in central Israel $(31^{\circ}59'N 34^{\circ}49'E)$. Additionally, a single sample of *M*. nicaeensis was harvested along with five other edible greens in a cultivated field at Sandala, North Israel (32°31'N 35°19'E). The list of focal species include therefore the following seven species: Hubeza, M. nicaeensis All., Spinacia oleracea L., Cichorium endivia L., Tetragonia tetragonioides Pall., Beta vulgaris L., and Rumex sp. We kept the leaves in a cooler to prevent fungal infestation.

Phytochemical analysis of elements in the leaves. Leaves were harvested at the base of the leaf, not including the petiole, massed for fresh weight (FW) and dried in an oven (60°C for 72 hours). Dry samples were weighed again to determine dry weight (DW) and water content (WC) then ground into a powder using an electric grinder. 100mg of each sample dried powder was used for macro- and micro- element analysis. Macroelements (N, P, K) were analysed using protocol

for digestion by sulfuric acid (H₂SO₄) in digestion glass tubes using a heat block (Bar-Tal et al., 2004). Digestion was completed at 250 °C for 30 minutes, samples were stirred and digestion continued for another 30 minutes before addition of H₂O₂. The resulting extracts were diluted with 98 ml distilled water to make 100 ml solution. Nitrogen (as NH₄⁺) and Phosphorus were analyzed in automated discrete photometric nutrient analyzer (GalleryTM Plus, Thermo Fisher Scientific). The values for NH₄⁺ were multiplied by 0.77 to get values for total N (National Center for Biotechnology Information). Protein was estimated by multiplying N concentrations by a factor of 6.25 (Mariotti, Tome, & Mirand, 2008). These extracts were also used to test Potassium and Na using a Flame Photometer (Model 410 Flame Photometer produced by Sherwood Scientific Ltd, Cambridge, UK). Additional elements were analyzed using protocol for digestion by nitric acid using a heat block (Zarcinas, Cartwright, & Spouncer, 1987). After digestion, the samples underwent specific elemental analysis using an atomic absorption spectrometer (Perkin-Elmer Analyst 400, Norwalk, Conn.).

Data analysis. The probability of fit to normal distribution was tested by overview of residuals plots and Shapiro–Wilk test. In the case of a Gaussian distribution, the means of the treatments were compared by a simple T-test. The datasets deviating from normal distribution were compared by a non-parametric multiple comparison tests after Wilcoxon to locate the differences. We refer to a P value ≤ 0.05 as statistically significant. All the statistical analysis performed by R open source (Team, 2019).

Results and Discussion

First, we present here typical images of the *Hubeza* plants - *M. nicaeensis*, and *L.cretica* (Fig. 1). The photos are illustrating how similar the species appear in the field before bloom, suggesting that when harvested wild – several species are highly likely to mix. This likelihood is justifying the generic use of "*Hubeza*" as the target of this study, rather than identified Malvaceae species in controlled conditions. At the time of sampling, specific *Hubeza* species could not be identified. Second, represented in photos are seven species that were studied in this research: we compared wild *Hubeza* plants to six species, used as a comparable resource for commercially grown green edible leaves (Fig. 2).



Figure 1. Green *Hubeza* leaves - *M. nicaeensis* and *L.cretica*. A) Mature blooming shoots of *M. nicaeensis* (left, redish stem) and *L.cretica* L. (right, green stem); B) Wild *Hubeza* patches, ruderal plantation; C) *M. nicaeensis* flowers (note the 3-part narrow epicalyx); D) *M. nicaeensis* leaf surface; E) *L.cretica* flowers (note the 3-part wide joined epicalyx); F) *L.cretica* leaf surface.

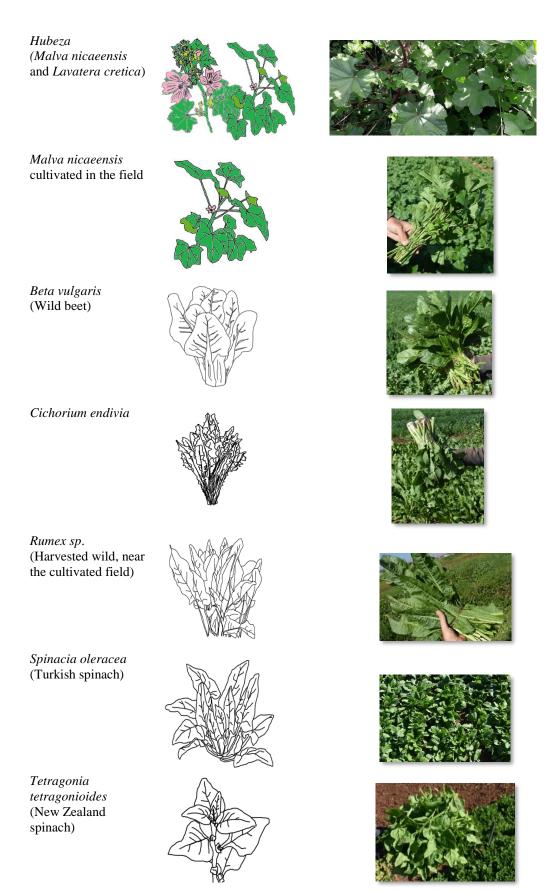


Figure 2. Green edible leaves grown in a commercial small farm in Israel. Photos taken by Oren Shelef. The icons that we created illustrate each species as a visual morpho-type – we used these illustrations in figure 3.

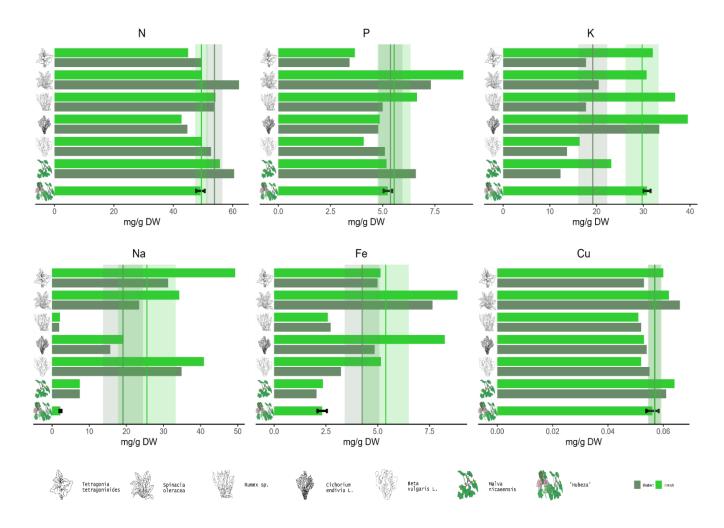


Figure 3. Concentrations of nutrients found in wild Hubeza are compared to those found in six species of cultivated green leaves. Horizontal bars represent the values per each species. Concentrations are all given in mg per gram dry weight (DW) of the sample. Green bars represent dried fresh leaves. Grey bars denote material dried from fresh leaves, which were soaked in boiled water for 10 minutes, to demonstrate the common practice for using green leaves in the kitchen. Vertical pale bars represent the average \pm standard error of the 6 species of green cultivated greens (n=6, one sample per species). Hubeza leaves are represented with error sign, denoting \pm standard error (n=51).

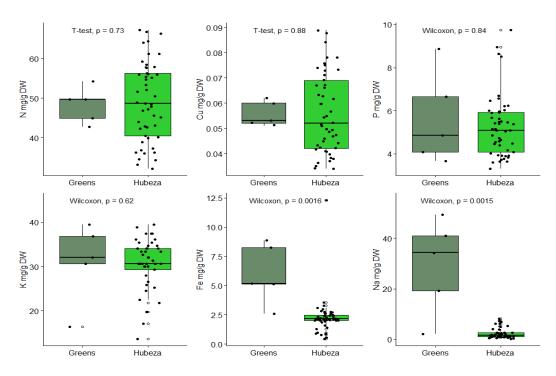


Figure 4. Difference between concentrations of nutrients (mg/g DW) found in *Hubez*a as compared to cultivated green leaves presented in a boxplot graph. Statistical tests and their *P* values, which were carried out based on the nativity of the residuals, mentioned at the top of each plot. "Greens" include 5 green leaves from the field, *M. nicaeensis* excluded. "Hubeza" include 51 accessions of wild populations of *M. nicaeensis* and *L. cretica*.

The average percentage of N, P, K, and Cu in the dry weight of *Hubeza* did not significantly differ from the averages of the cultivated greens (Fig. 3,4). Results show that the Na concentrations in Hubeza were significantly lower than all other greens, suggesting that Na is not accumulated extensively in Hubeza leaves. While this trait is advantageous for human nutrition, it may reveal that Hubeza are not tolerant to saline conditions, and this should be taken into account when planning for cultivation, irrigation, and fertilization. Na is desired in smaller amounts and Hubeza contains less than a fourth of the cultivated greens tested. The average Fe in Hubeza leaves was only half as much as the average among the other greens, 0.24% Fe by dry weight versus 0.54% Fe average. However, the percent by fresh weight was nearly the same (0.047% vs 0.042%). The results for the single known sample of M. nicaeenis was consistent with these averages for Fe in Hubezah. As the cultivated Malva nicaeensis showed a similar low level of Fe, this may suggest that *Hubeza* is not rich in Fe in comparison to other green leaves. This, however, should be measured in controlled conditions, with calculation of mass balance, and yield per area, to get a clearer conclusion. High levels of N in the leaves of a nitrophilic plant, may translate into high levels of nitrates, which are known as anti-nutritive to humans, and at some thresholds detrimental to herbivores (Barros et al., 2010; Vermunt & Visser, 1987). Multiplying the N levels of *Hubeza* by a 6.25 factor (Mariotti et al., 2008) results in high protein levels with an average of 60.8±2.0 mg/g FW, or 306.5±8.8 mg/g DW. These high levels of estimated total protein may be an overestimation driven by the high levels of nonprotein N. however, if we consider the protein levels as reliable, *Hubeza*'s N content translates into 6 g Protein per 100 g of fresh leaves. This means that a large 50 g bunch (1-2 handfuls) of Hubeza spp. leaves would satisfy 6% of the 50 g recommended daily protein consumption (Protein, FDA Factsheet). Hubeza had about 25% more P than Spinacia oleracea, the leader in P among the culitvated greens. The average K was similar to the other greens tested, and 30g serving of leaves would still supply 78% of an adults recommended daily consumption (FDA vitamin and mineral chart). The concentration of Cu in *Hubeza*, which is an antioxident and helps metabolize the Iron, also surpasses or equals to the other greens in concentration (FDA vitamin and mineral chart). According to the recommended daily intake values provided by the FDA, 100 g fresh Hubeza provides 12% protein, 10% P, 260% K, 264% Fe, 55% Cu, and 1.7% Na (FDA vitamin and mineral chart). This supports *Hubeza*'s nutritional importance. nicaeenis showed higher levels for N, protein, and Cu than the other greens. A bunch of M. nicaeenis boasted 41 mg Protein per g of fresh weight, higher than the others but lower than the 60.8 mg average for *Hubeza*. M. nicaeenis was also among the highest in other nutrients: second to Spinach in Phosphorus, third to Cichorium endiva and Rumex sp. in Potassium. It was second to last in Iron and Sodium. The inconsistent variance in *Hubeza* values could be explained by the manner of sampling wild populations. The sample size for the cultivated greens was much smaller than that of Hubeza. Additionally the Hubeza leaves were harvested individually from wild plants and the cultivated greens were tested as received from the

farmer in bunches including stems. As such, the fresh water weight of the stem was included for the greens but not in the *Hubeza* leaves. However, since most nutrients are concentrated in the leaves, the dry weight needs to be considered as well. Our results for fresh *Hubeza* and Spinach (*Spinacia oleracea*) were higher in all aspects than the results recorded for fresh raw spinach from the USDA National Nutrient Database for Standard Reference. Our results show that in comparison to other commercially grown green leaves *Hubeza* have sufficient nutritional potential to be considered as a food crop.

Conclusions and Future Aspects

The nutrient results observed for *Hubeza* were comparable to similar greens grown for culinary uses making it a very good candidate for a food crop. Additionally, the nutrient analysis of the dried *Hubeza* leaf may support its use as a health supplement (leaf powder). To complete the examination of Malvaceae leaves as a novel food crop, it's important to add more nutrition factors analysis, including other minerals as Ca and Mg as well as vitamins, anti-oxidants, polyphenols, and amino-acids. It is also important to examine for anti-nutrients such as oxalate and nitrate. High concentrations of nitrates could have detrimental effect on cattle, so the impact of nitrophilic plants should be taken into account. In the future we will conduct open field experiments to test its viability to be grown in classical agricultural settings. Based on preliminary germination experiment, which exhibited 0-8% germination, we suspect that Malvaceae has dormant seeds, hence the first agronomic aspect which we are planning to test is the propagation barrier. We will test for the optimal conditions for seed sprouting temperature, and dormancy removal techniques. Finally, we will grow the plants in an open field experiment to test the plausibility of growing green leaves of specific Malvaceae sp. for commercial use.

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