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Comparative Study of Fresh and Various Drying Methods on the Proximate and Phytochemical Analysis of *Urtica dioica*

Ridhima Singh[1], Mansi Chaudhary[1], Ekta Singh Chauhan[2]

[1]Research Scholar, [2]Associate Professor;
Department of Food Science and Nutrition, Banasthali Vidyapith, Tonk, Rajasthan-304022

Corresponding Author: Ekta Singh Chauhan

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ABSTRACT

Urtica dioica, a highly nutritious medicinal herb that grows naturally, requires no maintenance. This herb is rich in protein, calcium, and iron, as well as countless bioactive compounds making them supportive to combat various diseases, particularly diabetes, anemia, cancer, and hypertension. The current study was designed to determine the proximate and phytochemical analysis of fresh leaves and dried leaves of *Urtica dioica* by using different methods: sun-drying, shadow-drying, and oven-drying. The experiments were performed by the standard procedure of the Association of Analytical Communities. The sun-drying and oven-drying leaves of *Urtica dioica* were found to have a similar and maximum concentration of protein as compared with the fresh and shadow-drying leaves. Furthermore, calcium and iron content were higher and found significantly maximum ($p<0.05$) in sun-drying leaves as compared to fresh, shadow-drying, and oven-drying leaves. Qualitative phytochemical analysis showed the presence of bioactive compounds in both fresh and dried leaves of *Urtica dioica.* The presence of alkaloids, flavonoids, saponins, tannins, and terpenoids was confirmed in the ethanolic extract in the current study. Cardiac glycosides and anthocyanin were not present in the leaves of *Urtica dioica*. Meanwhile, the current study confirmed bioactive compounds are not sensitive to the drying process of leaves. *Urtica dioica* is an excellent source of nutrients and phytochemicals used as a fresh green herb as well as in dried form for the prevention of various nutrient deficiency disorders. Hence, it should be added as a nutritional supplement.

Keywords: *Urtica dioica*, Sun-drying, Shadow-drying, Oven-drying, Proximate, Phytochemical.

INTRODUCTION

Medicinal herbs have been used for thousands of years due to their excellent potential in herbal treatment without any side effects. Medicinal herbs are generally recognized as a valuable asset, easily available, and affordable for medical practice. As reported by World Health Organization (WHO), 80% of the total world's population depends on medicinal herbs, which require plant extracts as active ingredients. The commercial application of medicinal herbs in various sectors such as herbal remedies, herbal foods, herbal drinks, nutraceuticals, cosmetics, perfumes, and many products in daily use. [1] However, some herbs play an active role in therapeutic efficacy and exhibit nutritional value in the diet of human beings, which in turn can be helpful in particular human diseases. [2] In spite of this fact, herbs are excellent sources of protein, vitamins, minerals, phytochemicals, and antioxidants which will reduce the incidence of many chronic diseases. [3] Furthermore, according to WHO estimates present annual demand for medicinal herbs is $14 billion and it will be $5 trillion in 2050. [4] Moreover, the

addition of herbs and their functional ingredients in food products is a better option for strengthening medicinal outcomes. Basically, the biologically active compounds which enhance the medicinal effects of herbs are flavonoids, saponins, tannins, alkaloids, anthraquinones, and phenolic compounds. [5]

Urtica dioica (UD) is utilized globally in traditional medicine for various health ailments such as anemia, allergies, arthritis, obesity, and wound healing. UD is a perennial herb growing extensively in Europe, Asia, North America, and North Africa. In South America, soldiers and tribes used fresh UD to get relief from pain and other health ailments. [6] Previous studies reported that UD is used as a vegetable, in salads, and juices, and also for traditional home remedies. [7] UD has been used for the treatment of colds, coughs, cuts, and wound healing. It is also used as a diuretic for the prevention of high blood pressure in India where it is commonly known as bichhu ghas. [8] Furthermore, in Dewal village in Uttarakhand, leaf juice is used to treat boils, blisters, and epilepsy. In Italy, leaves are beneficial to treat rheumatoid arthritis, and gastrointestinal disorders. [9] The UD leaves are reported to have numerous medicinal activities such as anti-hypertensive, anti-diabetic, anti-cancer, anti-atherosclerotic, anti-asthmatic, anti-ulcer, anti-dandruff, anti-colitis, and anti-microbial. [10] Traditionally, UD leaves are used for the treatment of polycystic ovary syndrome. [11] A number of biologically active compounds such as tannins, flavonoids, quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside, sterols, and organic acids have been present in UD. [12] Flavonoids such as kaempferol-3-O-rutinoside, quercetin-3-O-rutinoside, and isorhamnetin-3-O-glucoside present in UD are effective in vitro immunomodulatory effects. [13] The sterols, triterpenes, lectin, polyphenols, and flavonoids were isolated from UD reducing blood glucose levels and exhibiting anti-diabetic effects. [14] UD were screened for their anti-hepatoprotective effect due to the presence of polyphenol oxidase. [15] The objective of the current research was to evaluate the nutrient and phytochemical analysis of fresh leaves and dried leaves by using different methods: sun-drying (SD), shadow-drying (SHD), and oven-drying (OD) of herb UD.

MATERIALS AND METHODS

Chemicals

Dragendorff's reagent (44578), Mayer's reagents (MHS16), potassium thiocyanate (207799), and bromocresol green (114359) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Potassium sulfate, copper sulfate, sulphuric acid, sodium hydroxide, boric acid (MB007), methyl red, ethanol, potassium permanganate, hydrogen chloride, potassium persulfate, ferrous ammonium sulfate, ammonium oxalate, ammonium sulfate, sodium oxalate, oxalic acid, ammonium hydroxide, ferric chloride, methanol, and chloroform were purchased from SRL (Mumbai, India). The rest of the additional chemicals were of analytical grade used for the study.

Sample preparation

The UD fresh leaves were obtained from Patanjali Herbal Botanical Garden, Uttarakhand, India. UD fresh leaves were cleaned under running tap water to take out all the dust, mud, and unwanted particles. The UD fresh leaves are separated into four parts. One part of the herb was used as a fresh sample, while the other three were dried under the sun, shadow, and oven for the study. Dried leaves were stored in an air-tight container at room temperature until laboratory analysis, which was performed under one month after harvest.

Proximate analysis

Proximate analysis of fresh leaves and dried leaves of herb UD was carried out according to the procedure given by the Association of Official Analytical Chemists (AOAC). [16] For moisture content analysis, drying the samples in the hot air oven at 105 ˚C until a constant weight was obtained. Ash content

was determined by using a muffle furnace for 12 h at 550 ˚C. The protein was estimated through micro Kjeldahl's distillation method. [17] The crude fiber of the samples was determined by method. [18] Furthermore, macro and microelements calcium (Ca) and iron (Fe) were analyzed in fresh and dried leaves of the UD. The Atomic Absorption Spectroscopic standard method was used for determining the digested samples by using a BUCK Scientific 200A apparatus for Ca and Fe. [19]

Phytochemical analysis

The amount of 1 g of fresh leaves and dried leaves of UD were boiled in 16 ml of double distilled water till the quantity was reduced to 4 ml. For further study, the soxhlet extraction method was used for extract production. Ethanol was used as a solvent. The above-prepared extracts were used to examine whether the bioactive compounds such as (alkaloids, flavonoids, cardiac glycosides, saponins, tannins, terpenoids, and anthocyanin) in all the
leaves samples were present or absent through the usage of a standardized method. [20, 21]

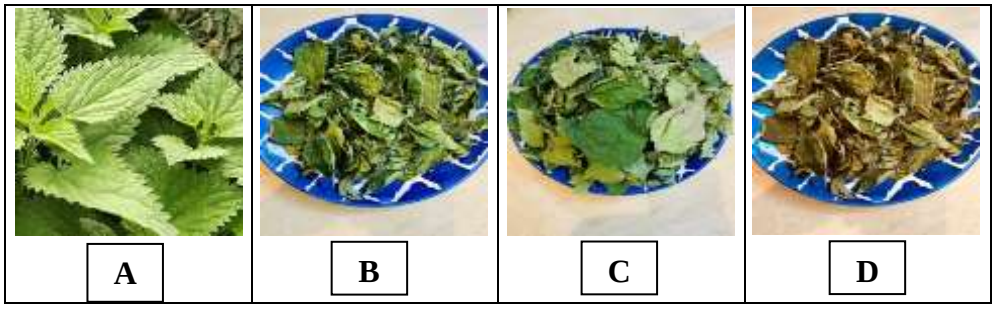

Figure 1. Leaves of *Urtica dioica* [A] Fresh, [B] Sun-drying, [C] Shadow-drying, [D] Oven-drying

STATISTICAL ANALYSIS

All the results were expressed as mean ± standard deviation. Statistical analysis was performed by using analysis of variance (ANOVA) GraphPad software (GraphPad Prism 8.0.2). Intergroup comparisons were done by Turkey's post hoc companions. Differences with ($P<0.05$) were considered significant as determined by the least significant difference (LSD).

RESULTS AND DISCUSSION

Proximate composition

(Figure 2) exhibit the proximate composition of fresh leaves and dried leaves of UD. The moisture, ash, protein, crude fiber, calcium, and iron were significantly affected by the drying methods. Fresh leaves and dried leaves have an appreciable amount of different nutrients. This means that UD is an excellent source of nutrients and have the capacity to combat various nutrient-related illness. The moisture content was higher in fresh leaves as compared to the SD ($P<0.05$), SHD ($P<0.05$), and OD ($P<0.05$) leaves respectively. Ash content which acts as an indicator of mineral composition in plants was higher in OD than in fresh leaves ($P<0.05$), and those dried with SD ($P<0.05$), and SHD ($P<0.05$). Protein and crude fiber content were higher in dried leaves as compared with fresh leaves value significantly ($P<0.05$). Ca and Fe were higher in SD leaves as compared with the fresh leaves, SHD, and OD leaves ($P<0.05$) respectively.

The content of moisture in fresh leaves was found to be elevated as compared with the dried leaves of UD in the current study. In dried leaves of the current study, moisture content was higher in SD as compared with SHD and OD leaves respectively. Studies were conducted on UD fresh leaves, [22] dry leaf powder, [23] and OD leaves. [24] The content of moisture in UD leaves indicates that moisture content is higher in fresh leaves as compared with dried leaves. In

another study, moisture content was observed similar in UD fresh leaves. [25] The content of moisture in dried leaves was reported almost similar (7.04%) to the present study of UD found on the SD leaves. [26] Another study conducted on the moisture content of UD in freeze-drying leaves was almost similar (4.96%) to the SHD leaves of the present study. [27] The frozen chopped leaves were reported less moisture content (79.8%) as compared with fresh leaves of UD. [28]

The content of ash in the current study suggested that dried leaves had higher ash content as compared to fresh leaves. The dried leaves of the current study showed higher ash content in OD leaves as compared with SD and SHD leaves. The content of ash in OD leaves was higher compared to the present study. The ash content in fresh leaves was (3.06%) as reported by [29] similar to the present study of fresh leaves. Ash content in a similar study was (2.1%) reported by. [22] The ash content of dried leaves was found to have (18.86%) as reported by [30] almost similar to OD leaves. The study described by [26] was reported to have ash content in UD dry powder similar to the SD leaves of the present study. A similar amount of ash content was found in dried leaves (19.75%) similar to the OD leaves. [31]

The protein content in the current study showed an increased protein content in dried leaves as compared with the fresh leaves. In dried leaves, protein content was lower in SHD leaves as compared to SD and OD leaves. Although, protein content was almost the same in both SD and OD leaves respectively. A similar amount of protein was found in fresh leaves (4.3%) as conducted by. [32] A fair amount of protein was reported in dry leaf powder (33.8%) as reported by. [8] The protein content in fresh leaves was (4.74%) as reported in the present study. [33] In dried leaves powder, the protein content was low (17.36%) when compared with the current study depicting protein content higher in dried leaves. [34] The protein content in freeze-dried powder of UD leaves (5.28%) was much lower. [35] The amount of protein in SD (29.53%) and SHD (28.37%) was almost the same as in the current study which confirms a similar amount of protein in both SD and SHD leaves. [36] Protein is a very important building block of bones, muscles, and tissue. The standard protein content of pulses is around (25%) [37] as compared with the UD dried leaves, the protein content values are approximately (30%) as shown in (figure 2). Furthermore, results indicate that protein content is significantly less affected by the drying methods.

The crude fiber in fresh leaves was very low as compared with the dried leaves in the current study findings. In dried leaves, crude fiber was higher in OD leaves as compared with the SD and SHD leaves respectively. The crude fiber in fresh leaves (2.9%) was less in comparison to the current research indicating a slightly higher content of crude fiber in fresh leaves. [38] The crude fiber content in fresh leaves (4.6%), freeze-dried (27.5), and OD leaves (26.3%) are reported. [24] The crude fiber content was low (14.8%) in dried leaves [39] compared with the other study (19.62%). [31] The crude fiber in a good amount (31.65%) was found in the roots of the UD. [30]

Minerals like Ca are very useful for proper growth, and for building strong bones, muscles, and teeth. [40] Ca content in dried leaves was higher as compared with the fresh leaves in the present study. Also, in dried leaves, SD leaves had a higher content of Ca as compared with the SHD and OD leaves. The Ca content in fresh leaves (2136%), freeze-dried (2283%), and OD leaves (2065%) was present in fair amounts. [24] The Ca content in fresh leaves was (853%) respectively. [13] Another study showed Ca content in raw leaves (3166%), and in cooked leaves (1452%) which was good in amounts. [41] Higher Ca content in SD (4710.96%), SHD (4222.65%), and microwave-drying (4417.87%) was reported in another study. [36] A very less amount of Ca was reported in dried leaves (2.63%), stem (1.06%), and root (0.89%). [42] UD is a

rich source of Ca as depicted by the present study. UD Ca content has the potential to supply a considerable amount of Ca for good health and wellness if incorporated into the diet. [43]

Fe plays a significant role in the human body. It is an important element that takes part in various metabolic processes, helps to synthesize various hormones, and prevents anemia in living organisms. [44] In the present study, Fe content was higher in dried leaves in comparison with the fresh leaves. Also, in dried leaves, SD leaves had a higher content of Fe as compared to SHD and OD leaves. A study showed lower content of Fe in fresh leaves (1.64%). [25] In another study Fe content in fresh leaves (16.7%), freeze-dried (17.9%), and OD leaves (17.6%) respectively. [24] Fe content in various drying processes of leaves is SD (25.7%), SHD (26.3%), and microwave-drying (23.9%) which is quite similar to the present study.[36] In various studies, an excellent amount of Fe (227.8%, 99.9%, 18.2%,13%, and 7.7%) was present in dried leaves. [23, 45, 46, 13, 47] The Fe content in dried leaves (29.6%, and 30%) is more or less similar to the present study of SD leaves. [48, 32] As from the present result, UD is an excellent source of Fe and must be included in the diet in its fresh or dried form.

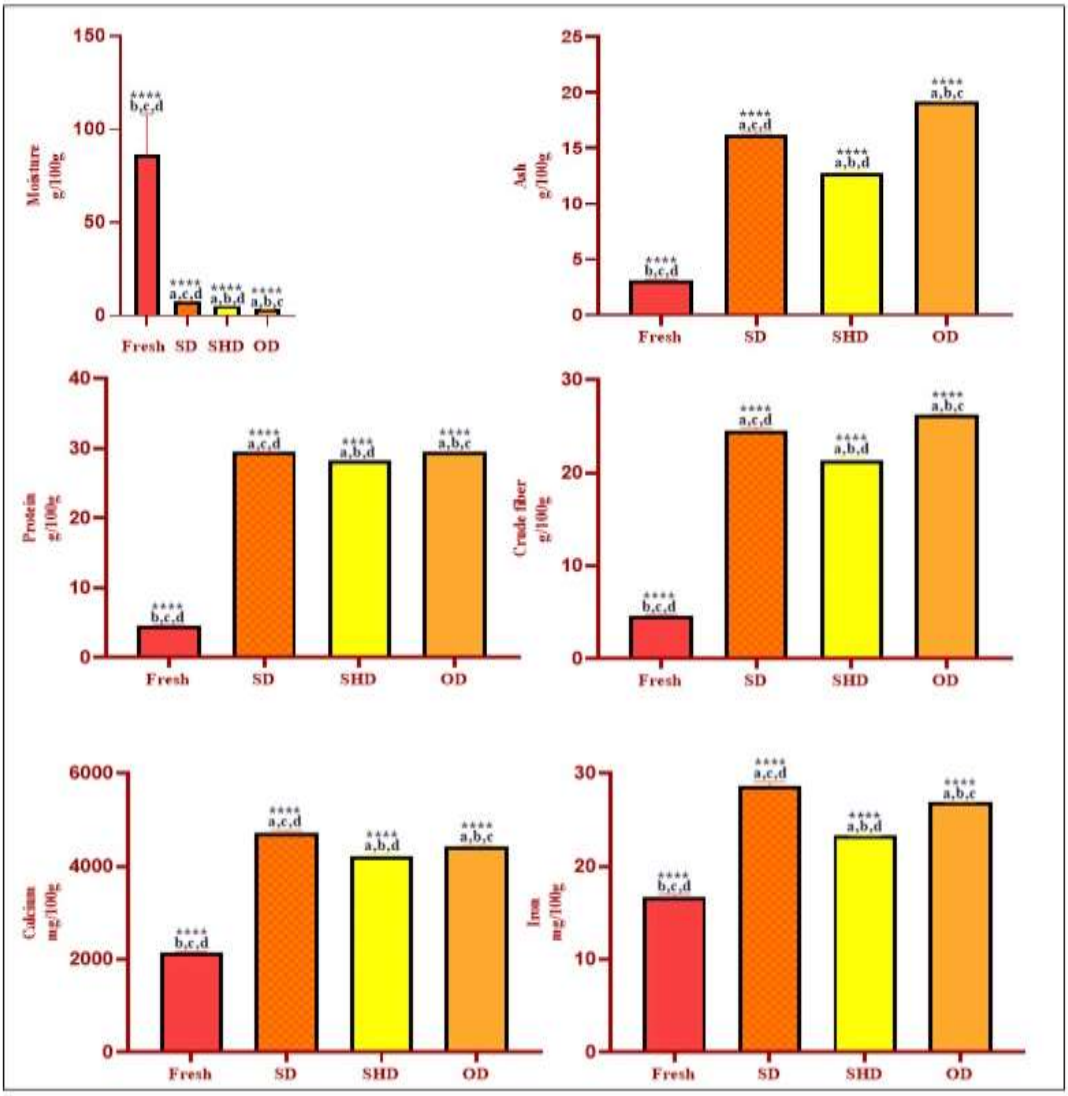

Figure 2. Proximate composition of fresh and dried leaves of *Urtica dioica*. Leaves were dried with different methods: sun-drying (SD), shadow-drying (SHD), and oven-drying (OD).
Results are shown as mean ± standard deviation. Results are significant at ****p<0.05. a-compared to fresh, b-compared to SD, c-compared to SHD, and d-compared to OD.

Phytochemical screening

Qualitative phytochemical analysis revealed the presence of various bioactive compounds in the fresh and dried leaves of UD. Various pharmacological properties are shown due to the presence of biologically active compounds (Table 1). Ethanol extract from fresh and dried leaves of UD was used to examine the presence of alkaloids, flavonoids, cardiac glycosides, saponins, tannins, terpenoids, and anthocyanin. The current study suggested that alkaloids, flavonoids, saponins, tannins, and terpenoids were present in both fresh and dried leaves of UD. Cardiac glycosides and anthocyanin were absent in all the leaves samples. Furthermore, the result clearly indicated that bioactive compounds are not sensitive to the drying process of leaves. UD leaves are reported to have excellent amounts of bioactive compounds in their leaves, roots, and stems. [49, 50, 30, 51]

Table 1. Phytochemical screening of fresh and dried leaves of *Urtica dioica*. Leaves were dried with different methods: sun-drying (SD), shadow-drying (SHD), and oven-drying (OD).

S. No.	Phytochemicals compounds	Ethanol			
		Fresh	SD	SHD	OD
1.	Alkaloids	+	+	+	+
2.	Flavonoids	+	+	+	+
3.	Cardiac glycosides	-	-	-	-
4.	Saponins	+	+	+	+
5.	Tannins	+	+	+	+
6.	Terpenoids	+	+	+	+
7.	Anthocyanin	-	-	-	-

+ represents presence; - represents absence

Alkaloids have dynamic medicinal properties such as anti-hypertensive, anti-inflammatory, anti-plasmodic, anti-psychotic, insecticidal, and hepatoprotective activity. [52, 53] The alkaloids content was found to be present in fresh leaves. [33] The SHD-dried leaves were found to have alkaloids content in the alcoholic extract and a complete absence in the watery extract. [54] In another study, alkaloids were present in ethyl acetate extract of air-dried leaves. [55] The alkaloids were depicted higher in UD leaves as compared with the Nootka rose reported in another study. [56] Flavonoids in UD leaves displayed a number of pharmacological activities including wound-healing, [57] reduces cholesterol levels, [58] anti-cancer, [59] and antiaggregant effects. [60] In water extract, flavonoids content was present in air-dried leaves. [61] In another study, SHD-dried leaves in ethanol extract were found to have flavonoids in different samples of Dehradun, Rudraprayag, and Pauri. [62] The flavonoids content of dried UD leaves powder in acetone, ethanol, and aqueous extracts tested positive. [63] In fresh leaves and stalks, flavonoids were found to be higher in ethanolic extracts. The presence of flavonoids in the leaves of the UD turned out to be beneficial in various industries such as food, cosmetics, phytomedicine, and textiles. [64, 65] The saponins possess several health benefits such as hypoglycemic, hypolipidemic, antioxidant, anti-asthmatic, anti-microbial, and anti-hypertensive activity. [66] The SHD-dried leaves, fruit, and root in methanolic extract revealed the presence of saponins content. [67] In another study, air-dried leaves in water extract reported the presence of saponins. [61] In both aqueous and ethanolic extracts, SHD-dried leaves were found to have saponins content. [62, 68] Furthermore, both raw and cooked leaves of UD were reported to have saponins content in good amounts. [41] Tannins had a wide range of health benefits in the human body such as wound healing, anti-diabetic, anti-cancer, anti-microbial, anti-viral, and anti-oxidant effects. [69] Dried powder of UD leaves depicted the presence of tannins content. [70] Both watery and alcoholic extracts of SHD-dried leaves revealed the tannins content. [54] The number of tannins was higher in fresh UD leaves as compared with *Urtica urens* leaves. [71] In another study, UD leaves

powder had a higher content of tannins compared with
barley and wheat flour. [26] The ethyl acetate extract of air-dried leaves was reported to have tannins content. [55] SHD-dried leaves were found to have tannins content in various locations of Uttarakhand such as Dehradun, Pauri, and Rudraprayag. [62] Terpenoids have recorded immense therapeutic potential such as nephroprotective, cardioprotective, hepatoprotective, and antioxidant activity. [72] In the methanolic extract, the presence of terpenoids was confirmed in the leaf, fruit, and root. [67] In the SHD-dried leaves in ethanolic extract, terpenoids was present in different samples of Dehradun, Rudraprayag, and Pauri. [62]

CONCLUSION

This study enlarges and complements the literature knowledge on the proximate and phytochemical properties of UD leaves. UD is well known medicinal herb with no side effects. This herb well grows in nature without any human effort and can be used as food as well as medicine. The current study aided the information about the proximate composition and phytochemical screening of both fresh and dried UD leaves which might be the prime factor for their pharmacological effects. Furthermore, this research provides empirical evidence that the dried leaves yield more nutrients as compared with the fresh leaves. The findings of the current study showed that protein content was higher and similar in both SD and OD leaves. Additionally, Ca and Fe were higher in SD leaves. This study confirms that UD leaves are a good source of nutrition and that SD is the best method for drying the leaves. It can be used in fresh forms where available naturally, and in dried forms to keep its nutrients intact to the maximum value. The significance of the drying process apart from this, it may lead to the development of novel nutrient-rich food products for the prevention of nutrient deficiency diseases.

Abbreviations

World Health Organization	WHO
Urtica dioica	UD
Sun-drying	SD
Shadow-drying	SHD
Oven-drying	OD
Association of Official Analytical Chemists	AOAC
Calcium	Ca
Iron	Fe
Analysis of variance	ANOVA
Least significant difference	LSD

Declaration by Authors

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