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Effect of Drying Methods on Phytochemicals, Antioxidant Activity and Total Phenolic Content of Dandelion Leaves

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Abstract *Taraxacum officinale* is globally used both as a vegetable and as a herb for medical and therapeutic purposes; hence the need to investigate its phytonutrients. The aim of this study was to evaluate the effects of different drying methods (hot-air drying, solar drying and freeze-drying) on the phytochemicals as well as total phenolics content and antioxidant capacity of dandelion leaves. The fresh dandelion leaves had high contents of total phenolics (7.78 mg GAE/ g) on dry weight basis. They exhibited high antioxidant activity (397.94%, %inhibition of DPPH) measured by DPPH assay. Drying methods caused a significant decrease in total phenolics and antioxidant capacity of dandelion leaves. Drying by freeze drying and solar drying had the lowest adverse effects on antioxidant capacities of dandelion leaves while drying by hot-air at 60ºC cannot be a competitive process for preserving antioxidants and antioxidant capacity of dandelion leaves. The changes in the antioxidant capacity due to the drying methods were positively correlated with the content of phenolics. Therefore, it can be suggested that special care should be taken when processing method is selected for the exploration of dandelion leaves.

Keywords: *DPPH, freeze-drying, percentage inhibition, gallic acid, Folin-Ciocalteu reagent, flavonoids*

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1. Introduction

Dandelion (*Taraxacum officinale*) is a perennial herb and belongs to the family Asteraceae [1]. Over the years, parts of the plant have been used in traditional medicine for various conditions. In Ghana, dandelion leaves are used as common vegetable, eaten as salad or used in soups and sauces, but a greater percentage of the population use Dandelion leaves for treating diseases such as heartburns, anaemia, abdominal disorders and liver diseases [1]. Dandelion leaves are often times a component of a traditional medicine formulation for the treatment of hypertension amongst Akans in Ghana. Various parts of the plant have been proven to be rich in phenolic compounds and antioxidant activities which contribute to its medicinal properties.

Martinez et al. [2] also reports that dandelion leaves have been used for centuries for relief and the treatment of many diseases due to the presence of several compounds such as sesquiterpenes, saponins, phenolic compounds, flavonoids and many more.

Medicinal herbs are usually dried to inhibit microbial growth and extend shelf life. Drying has the advantage of reducing transportation and storage costs but can result in changes in aroma and appearance which may affect plant quality. Drying has also been reported to cause reduction in plant bioactive compounds which may have beneficial health promoting properties such as antioxidant properties [3]. Though phytochemical constituents, antioxidant activities and total phenolic content of fresh dandelion leaves have been reported, little is known about the effect of different drying methods on phytochemicals, phenolic content and antioxidant activities of dandelion leaves.

This study therefore sought to provide information on the effect of different drying methods (solar, oven and freeze drying) on phytochemicals, phenolic content and antioxidant activities of dandelion leaves and determine the best method for drying the leaves.

2. Materials and Methods

Folin-Ciocalteu's phenol reagent, anhydrous sodium carbonate, gallic acid, aluminum chloride and sodium hydroxide were purchased from Sigma-Aldrich CO. Sodium nitrite, quercetin, 2.2-diphenyl-1- picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), potassium persulfate and 2,2´-azino-bis (3-ethylbenzothiazoline–6-sulfonic acid) diammonium salt (ABTS) were obtained from Sigma-Aldrich CO. Methanol, hexane and acetone (analytical grade) were from Sigma-Aldrich CO.

2.1. Source and Preparation of Dandelion Leaves Samples

Fresh dandelion (*Taraxacum officinale* L.) leaves were obtained from Horticulture Department of Faculty of Agriculture, KNUST, Kumasi, Ghana. Green leaves were manually separated from plant, washed with water and then drained and left to dry on a cheese cloth for 15 min at room temperature (35 ± 2°C). The moisture content of the fresh leaves was immediately determined according to the AOAC [25] method (number 934.01), and found to be 90.78 ± 0.03 g water per 100 g sample.

The samples were divided into equal parts and subjected to various drying methods; solar drying (for 48 h with temperature of solar dryer ranging between 57°C and 58°C), oven drying (Binder FD 115, Germany at 60°C for 12 h) and freeze drying (Vacuum freeze-drier, YK-118-50, Taiwan at -47°C to -53°C for 72 h). The dried samples were milled into powder and their moisture content determined [4]. The powdered samples were then stored in zip-lock pouches at 4°C prior to analysis.

2.2. Extraction of Antioxidant Compounds from Samples

10 mg each of ground sample was placed into separate beakers and 20mL of absolute methanol (Daejung chemicals and metals co. ltd.) added. Absolute methanol was used because it has been reported to more effective [5]. The mixture was sonicated for 30 min and then filtered using Whatman no.1 filter paper. The filtrates were then collected and used in the determination of antioxidant activity.

2.3. DPPH (2, 2-diphenyl-1-picrylhydrazyl) Assay

The antioxidant activity was determined using DPPH assay as described by Sochor et al. [6] and Sana et al. [7] with modifications. 2 mg of DPPH was dissolved in 100 mL methanol to obtain 0.002% DPPH solution. The absorbance of the control sample was obtained at a wavelength of 517nm. 10mg of each sample was dissolved in 20 mL of methanol to obtain a concentration of 500µg/ml. 1mL of each extract solution was pipetted into separate beakers and 3 mL of 0.002% DPPH added. The mixtures were well shaken and incubated in the dark for 30 min after which absorbance readings were taken at 517nm. The antioxidant activity was calculated using the formula:

$$\% \text{ Inhibition} = \left(A_0 - A_1 / A_0\right) \times 100$$

Where A_0 is the absorbance of the control (without the sample) and A_1 is the absorbance of the mixture containing the sample.

2.4. Total Phenol Determination

The Folin-Ciocalteu method is based on measuring the colour change from yellow to blue-black as a result of reduction of the Tungstate-molybdate mixture in the Folin-Ciocalteu reagent (Sigma Aldrich) by phenols present in the analyte solution [8].

2.4.1. Gallic Acid Stock Solution Preparation

Dry Gallic acid of 0.5 g weight was dissolved in 10 mL ethanol. This was transferred into a 100 mL volumetric flask and diluted to volume with distilled water.

2.4.2. Standard Calibration Curve for Phenol Analysis

In the preparation of a standard curve, 0, 1, 2, 3, 4, 5 and 10 mL of the Gallic acid stock solution were pipetted into separate 100 mL volumetric flasks and then diluted to volume with distilled water to give a standard Gallic acid solution of 0, 50, 100, 150, 250 and 500 mg/L, respectively. An amount of 0.1 mL of each standard Gallic acid solution was pipetted into a 10 mL volumetric flask and 6.0 mL distilled water added. A 0.5 mL Folin-Ciocalteu reagent (Sigma Aldrich) was then added. The solutions were well mixed and left to stand for 5 min after which 1.5 mL of 20 % sodium carbonate solution was added to each solution [9]. Finally the solutions were topped with distilled water to the 10 mL mark and mixed thoroughly. The resulting solutions were then incubated at room temperature for 90 min in the dark and the absorbance taken at 765nm using a UV-VIS spectrophotometer (Spectronic 20, Bausch and Lomb, Germany).

2.4.3. Sample Extraction and Analysis

For the extraction of the sample, 0.5 g each of dried powdered dandelion leaves was weighed into a beaker. 10 mL of 50% methanol was added and stirred; the sample extract was obtained by sieving. A 0.1 mL sample was pipetted into a 10 mL volumetric flask and 6.0 mL of distilled water added. This was followed by adding 0.5 mL of Folin - Ciocalteu reagent (2 N). It was well mixed and then left to stand for 5 min after which 1.5 mL of 20% sodium carbonate solution was added. The solution was made up to the 10 mL mark with distilled water and mixed thoroughly. The resulting solutions were then incubated at room temperature for 90 min in the dark. Afterwards, the absorbance readings were taken at 765 nm using a Synergy H1 microplate reader. Triplicate absorbance readings were taken for each sample. The total phenolic contents of each fraction were converted into milligram Gallic acid equivalents per gram dry weight of dandelion samples. Distilled water was used as blank in this experiment.

2.5. Phytochemical Screening

The methanol extracts were screened for major phytochemicals; alkaloids, saponins, tannins, flavonoids, steroids and terpenoids, according to standard methods described by Tiwari et al. [10] and Doughari [11] with slight modification.

2.5.1. Detection of Tannins

2 mL of each extract was mixed with a few drops of 0.1% ferric chloride. A brownish green or a blue black coloration indicated the presence of tannins.

2.5.2. Detection of Alkaloids

Extracts were dissolved individually in 2N HCl and filtered. 1 mL of each filtrate was treated with Mayer's reagent (Potassium mercuric iodide). Formation of

cream to yellowish precipitate confirmed the presence of alkaloids.

2.5.3. Detection of Flavonoids

2 mL of each extract was shaken with 1 mL of 1% NH_4Cl. A light yellow colour which became colourless on addition of dilute acid indicated the presence of flavonoids.

2.5.4. Detection of Terpenoids

This was done using Salkowski's Test. Extracts were treated with 2 mL of chloroform and after 3 mL of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration at the interface showed a positive test for terpenoids.

2.5.5. Detection of Steroids

This was done using Libermann Burchard's test. 2 mL of each extract was mixed with 2 mL of chloroform. 2 mL acetic anhydride and 2 drops of concentrated H_2SO_4 was added to the mixture along the side of the test tube. First red, then blue and finally green colour indicate the presence of sterols.

2.5.6. Detection of Saponins

Extracts were diluted in 10 mL of distilled water in a test tube and shaken for about 5 min. The test tubes were allowed to stand and a layer of foam that persisted confirmed the presence of saponins.

2.6. Statistical analysis

Data were expressed as means ± standard deviation (SD) of three replications, and one factor ANOVA was used for the statistical analysis using SPSS program (version 20 SPSS Inc., USA). The values were considered to be significantly different when $P<0.05$.

3. Results and Discussion

3.1. Moisture Content of dandelion Leaves

The moisture content of fresh dandelion leaves was found to be as high as 86.20% (Table 1). This was expected since most fresh leaves have very high moisture content. The moisture content of fresh dandelion leaves have been found to range from 86% - 87% [12,13]. This was reduced to 4 – 10% after subjecting the fresh leaves to the various forms of drying. Solar dried dandelion leaves had moisture content of 4.04%, freeze-dried leaves had 5.78% and that of oven drying had 10.23% (Table 1).

The moisture content of all the dried leaves were significantly different ($p<0.05$) from one other. This implies that the solar drying conditions employed resulted in more moisture loss from the leaves compared with the oven and freeze drying. The differences in moisture contents can be attributed to the length of time and temperature used in the different drying methods. Oven drying conditions (60 °C for 12 h) resulted in samples with higher moisture content than solar (57 – 58°C for 48 h) and freeze drying. The moisture contents of all dried samples obtained from the three drying methods were below 15% which is a critical factor in preservation of herbs [3]. The lower moisture contents (below 15%) of samples imply the dried samples will be more shelf stable than fresh leaves.

Table 1. Moisture content of fresh dandelion leaves after drying

Samples	Moisture content (%)
Fresh leaves	86.20 ± 0.10^a
Oven dried leaves	10.23 ± 0.70^b
Freeze dried leaves	5.78 ± 0.13^c
Solar dried leaves	4.04 ± 0.33^d

-Data is represented as mean ± standard deviation
-Values in same column with different superscripts are significantly different at 95% confidence level.

3.2. Phytochemical Screening of Dandelion Leaves

Phytochemical screening indicated the presence of terpenoids, steroids, tannins and saponins in fresh dandelion leaves (Table 2). Oseni and Yussif [14] reported the presence of these bioactive compounds in ethanolic extract of fresh dandelion leaves. Terpenoids, steroids, tannins and saponins were also detected in all the dried leaves (Table 2). The presence of these compounds after processing promotes their addition to our meals to increase the intake of these compounds. However, since the screening of these phytochemicals was qualitative, only their presence could be detected.

Although the quantities or concentration of these compounds were not detected besides the qualitative analysis done, the presence of these phytochemicals in dandelion leaves explains its ability to fight several diseases as has been reported [15]. For example, terpenoids have been reported to show biological activities against malaria, inflammation, cancer and a wide range of bacterial and viral infections [16]. Sharma and Paliwal [17] have also reported on the pharmacological effects of saponins which include anti-inflammation, anti-virus and their ability to kill tumour cells. Tannins have also shown to have anti-viral, antibacterial and anti-microbial effects [18]. Plant sterols have been reported to lower blood cholesterol levels and reducing the risk of cardiovascular diseases [19].

Table 2. Phytochemical screening of fresh and dried dandelion leaves

	Phytochemical compounds					
Sample	**Terpenoids**	**Steroids**	**Flavonoids**	**Tannins**	**Alkaloids**	**Saponins**
Fresh	+	+	-	+	-	+
Oven dried	+	+	-	+	-	+
Freeze dried	+	+	-	+	-	+
Solar dried	+	+	-	+	-	+

"-" represents the absence of the phytochemical
"+" represents the presence of the phytochemical.

3.3. Total Phenolic Content of Dandelion Leaves

Phenolic compounds have been found to be essential to plants as they aid in their defence against infection and injury (20). Dandelion has been found to be rich in phenolic compounds such as caffeic acid, coumaric acid, chlorogenic acid, chicoric acid and sinapic acid (21,22). The total phenolic contents of dandelion leaves were determined as gallic acid equivalence using the equation from the gallic acid standard curve. The highest phenolic content of 7.78 ± 0.26 mgGAE/g was recorded by fresh dandelion leaves. However, there was a decrease in concentration of phenolic compounds for dried dandelion leaves, with freeze dried leaves, solar dried and oven dried leaves having total phenolic contents of 4.31 ± 0.11 mg GAE/g, 4.19 ± 0.16 mg GAE/g and 2.95 ± 0.05 mg GAE/g respectively (Table 3).

Table 3. Total phenolic content of Fresh and dried Dandelion leaves (Dry basis)

Samples	Total phenolic content (mg GAE/g)
Fresh Dandelion leaves	7.78 ± 0.26^{a}
Freeze dried leaves	4.31 ± 0.11^{b}
Solar dried leaves	4.19 ± 0.16^{b}
Oven dried leaves	2.95 ± 0.05^{c}

-Data is represented as mean ± standard deviation
-Values in same column with different superscripts are significantly different at 95% confidence level.

The total phenolic content of fresh dandelion leaves have been reported to be 2.019 ± 0.40 mg GAE/g, fresh weight [22]. That obtained in this study was 7.78 ± 0.26 mg GAE/g (dry basis) and this can be expressed as 1.09 mg GAE/g fresh weight. This value is relatively lower than that reported by Harmankaya et al. [22]. And this variation in phenolic compounds may be due to differences in moisture content, extraction time and methods, geographical origins and harvesting season of samples [23[.

The results obtained also showed significant differences in phenolic content between fresh dandelion leaves and dried dandelion leaves. Drying caused reduction in phenolic content of dandelion leaves. Youssef and Mokhtar [24] reported that drying could cause changes in chemical structure of polyphenols or cause them to adhere together with other plant components (proteins) making their extraction, using available extraction methods, difficult; thereby resulting in lower recoveries than expected. Also, the cell structure may rupture as a result of thermal processing which may lead to migration of these components, hence their loss [24]. Various researchers have reported on the breakdown of bioactive compounds during thermal processing particularly oven drying. This degradation could be attributed to the activity of degradative enzymes such as polyphenol oxidases which have the ability to degrade phenolic compounds if activated by drying methods [3]. However, factors such as plant species and cell wall stability have been reported to influence the effect of drying on total phenolic content [25].

Freeze dried leaves had the highest phenolic content (4.31 mgGAE/g) as compared to other dried samples in this study (Table 3). Freeze drying has been proven to be an efficient method in retention of plant bioactive compounds and this is due to the fact that dehydration is at lower temperatures as compared to other drying methods [26]. There was however no significant difference ($p > 0.05$) in phenolic content of freeze dried and solar dried leaves. Oven dried leaves had the least phenolic content (2.95 mgGAE/g) and it was significantly different from the other dried leaves. This confirms that thermal processing causes the rupture of leaf cell structure which may lead to migration of phenolic components, hence their loss. Comparatively, the oven drying temperature was higher than all the other drying methods. Although the temperature of the solar drying is closer to that of the oven drying, this may not have been consistent throughout the drying period, since it depended on the sun's heat; as opposed to the oven drying which had consistent heat supply.

3.4. Antioxidant Activity of Dandelion Leaves

Antioxidant activities expressed as %inhibition of DPPH (dry basis) of Dandelion leaves ranged from 69.51% for oven dried to approximately four-folds (397.94%) for fresh leaves, in the order Fresh > Freeze > Solar > Oven. The highest percentage inhibition was found in the fresh dandelion leaves with the oven dried leaves having the lowest antioxidant activity (Figure 1). Fresh Dandelion leaves had 397.4%, which was significantly higher than 83.71% (freeze), 74.34% (solar) and 69.51% for oven dried samples. The respective antioxidant activities of the samples (Figure 1) represent the percentage at which the various samples inhibited the DPPH radical used in the assay. There were significant differences ($p < 0.05$) observed in antioxidant activities between all the dried samples and the fresh dandelion leaves.

Freeze dried leaves recorded the highest antioxidant activity among the dried dandelion leaves. This confirms what was reported by Mudau and Ngezimana [26] that freeze drying has the potential of retaining phenolic compounds and antioxidant activities of most samples as compared to other drying methods. There was however no significant difference ($p > 0.05$) between antioxidant activities of freeze dried and solar dried leaves. Oven dried leaves had the least antioxidant activities among dried samples implying that oven drying may not be an efficient method for drying dandelion leaves.

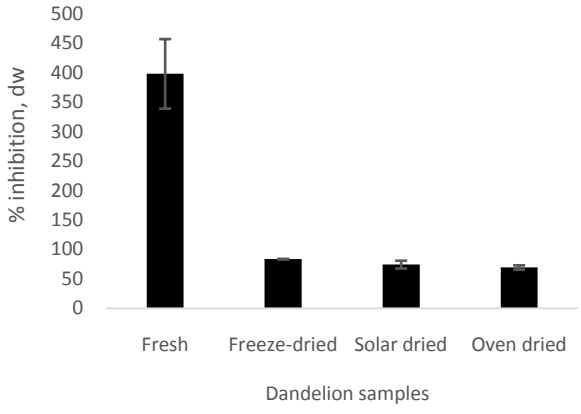

Figure 1. %Inhibition of DPPH (dry basis) by Dandelion extract at 500µg/ml-dw = dry weight

A direct correlation has been established between total phenol content and antioxidant activity [23,27]. In addition, phenolic compounds have been reported to have the potential of inhibiting free radicals [28]. The results obtained in this study showed a reduction in the concentration of total phenols in the dried samples as compared to fresh dandelion leaves (Table 3) and a similar trend was observed in antioxidant activity (Figure 1). This implies that the antioxidant activities of dandelion leaves may have been contributed to by the phenolic compounds in dandelion leaves. Further, a strong positive correlation ($r=0.876$, $p<0.04$) was observed between total phenolic content and antioxidant activity of dandelion leaves (Table 4).

Table 4. Correlation between the total phenolic content and antioxidant activity

		%inhibition	total phenol
%inhibition	Pearson Correlation	1	.876**
	Sig. (2-tailed)		.004
	N	8	8
total phenol	Pearson Correlation	.876**	1
	Sig. (2-tailed)	.004	
	N	8	8

**. Correlation is significant at the 0.01 level (2-tailed).

Oven dried leaves recorded the least antioxidant activity (Figure 1) and this could be explained by the possible degradation of most of the phenolic compounds during the drying process hence resulting in the least antioxidant activity. Dandelion leaves besides being eaten as any leafy vegetable is often used in traditional medicine for the treatment of several diseases. The phytochemicals, phenolic content and antioxidant activity of the leaves contribute to their medicinal properties. Preserving these properties after drying (common treatment to preserve leaves) is therefore very important. As a result, using freeze drying and solar drying for drying of dandelion leaves is better than using oven drying.

4. Conclusion

Total phenolic content and antioxidant activity of Dandelion leaves are significantly affected by the different drying methods. The different drying methods resulted in a decrease in total phenolic contents and antioxidant activities. Terpenoids, tannins, steroids, and saponins were identified in fresh leaves as well as dried dandelion leaves. Flavonoids and alkaloids were however not detected in all the samples. Freeze and solar drying retained most of the total phenolic content and antioxidant activity of dandelion leaves as compared to oven drying and will therefore be better drying methods for drying Dandelion leaves.

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