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Nalini Trivedi
Department of Food and Nutrition, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

Anuradha Dutta
Department of Food and Nutrition, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

Rita S Raghuvanshi
Department of Food and Nutrition, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

AH Ahmad
Department of Veterinary Pharmacology & Toxicology, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

Vinod Kumar
Department of Mathematics, Statistics and Computer Science, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

Anil Kumar
Department of Food Science and Technology, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

Corresponding Author:
Nalini Trivedi
Department of Food and Nutrition, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

Effect of drying techniques on nutritional composition and bioactive compounds of underutilized *Vernonia cinerea* (Whole plant) and *Cichorium intybus* (Leaves)

Nalini Trivedi, Anuradha Dutta, Rita S Raghuvanshi, AH Ahmad, Vinod Kumar and Anil Kumar

Abstract
The present study evaluated the effect of different drying techniques on macronutrient composition, ascorbic acid, phenolic and flavonoid constituents of underutilized whole plant of *Vernonia cinerea* and leaves of *Cichorium intybus* with potential metabolic and anti-inflammatory properties. The fresh herbs were dried in solar-dryer (35 ± 2 °C, 10 h) and oven in oven dryer (45 °C, 7 h), and milled into powder (particle size ≤ 500 µm) for further analysis. There was a significant decrease (p<0.05) in the moisture and carbohydrate content of solar dried samples as compared with oven dried samples with a corresponding increase in the protein, fiber and ash content in both the herbs. Fat content was decreased quantitatively in solar drying as compared to oven drying. Solar drying retained higher vitamin C content in both the plants (35.41 mg/100g and 108 mg/100g in *Cichorium intybus* leaves and *Vernonia cinerea* whole plant, respectively). Both the drying methods had significant effect on the phenolic constituents. Overall, solar drying emerged as a better technique for retaining macronutrients, ascorbic acid and bioactive compounds. Therefore, this method may be propagated for drying the herbs.

Keywords: Solar drying and oven drying, *Vernonia cinerea* (whole plant), *Cichorium intybus* (leaves), macronutrients, ascorbic acid, bioactive compounds

Introduction
Drying techniques are significant in food processing because they serve two purposes: preventing microbial growth and facilitating storage (Periche *et al.*, 2016) [29]. Drying can influence the initial quality in terms of appearance and the preservation of volatile components to a certain extent. Plant materials are dried using a variety of drying methods (Lin *et al.*, 2012) [19]. Solar and oven drying are two common drying techniques for fresh plant materials. For most smallholder farmers, solar drying is the most common, easiest, economical, and accessible method (Mujuka *et al.*, 2021) [25]. Solar drying, albeit inexpensive, has been observed to produce significant nutritional losses and takes more time to obtain the desired moisture content and weight (Putriani *et al.*, 2020) [31]. An alternative is oven drying which has shown to have minimal nutritional losses as of carotenes while improving the sensory attributes of color and tastes of plants (Managa *et al.*, 2020) [20]. Oven drying, on the other hand, is expensive and constrained by the availability of power supply (Zhang *et al.*, 2017) [36]. Various drying processes have different effects on the chemical components and antioxidant activity of medicinal plants (Periche, 2016) [29]. The impact of a specific drying process on raw quality retention is unpredictable and dependent on the compounds and plants in consideration. As a result, a comparative study of various drying procedures can reveal a large amount of information for enhancing the quality of goods as functional food ingredients or nutraceuticals. *Vernonia cinerea* (Asteraceae) is an herbaceous plant that grows in Africa, India and Asia. Little ironweed is the widespread name for this plant, which grows in open waste meadows, along roadsides, and in dry grassy areas. The presence of many bioactive compounds in *Vernonia cinerea*, such as flavonoids and glycosides, phenolic compounds, terpenoids, sesquiterpenes, amyrin and stigmasterols, gives it anti-inflammatory, analgesic, anthelmintic, and antioxidant activities. The juice from Vernonia cinerea leaf is given to children suffering from a urinary infection. Furthermore, the extracts are said to have no adverse effects when consumed. The plant is used to make herbal tea for smokers trying to quit smoking (Inpuron *et al.*, 2013; Prasopthum *et al.*, 2015) [13, 30]. Skin illnesses, coughs, bronchitis, asthma, malaria, cancer, gastrointestinal disorders, diuresis, aches and diabetes have all been treated using this

herb in the past (Prasopthum *et al.*, 2015; Youn *et al*., 2014) [30, 35]. Even though research on its nutritional and phytochemical content has been conducted, no literature has been published on the influence of different drying temperatures on the nutritional quality of the *Vernonia cinerea* whole plant.

Cichorium intybus L. is an herb commonly known as chicory. It is native to temperate regions and is grown in a variety of places across the world, including Europe, North America, and Asia. Chicory leaves are consumed as leafy vegetables. It contains several therapeutic properties, including anti-diabetic, antibacterial, anti-hepatotoxic, antioxidant, and wound-healing capabilities (Afzal *et al*., 2014) [2]. Chicory's therapeutic benefits have been related to many significant bioactive constituents found in the plant. The leaves have been shown to possess a significant amount of phenolics (190 2.03 mg/g dry matter) (Conforti *et al.*, 2009) [7]. The antioxidant potential of plant samples is significantly associated with the concentration of phenolic compounds, implying that chicory leaves are an excellent source of antioxidants (Cai *et al.*, 2004) [5]. Fresh chicory leaves, on the other hand, have a short shelf life due to enzymatic reactions and microbiological development. The most effective ways for maintaining the quality of fresh materials are thermal treatment, a preparatory processing procedure, and drying, a preservation technique (Lin L.Z. *et al*., 2012) [19].

There is currently a paucity of information on the application of drying technologies and their effects on *Vernonia cinerea* and *Cichorium intybus*. The majority of research focuses on the nutritional and bioactive components of *Vernonia cinerea* and *Cichorium intybus*, with little attention paid to the drying processes employed and their effects. Only a few researchers have looked at the effects of different drying techniques on different food applications. The goal of this study was to see how drying procedures affected the nutritional and bioactive substances in *V. cinerea* and *C. intybus*, such as total phenol, flavonoids, ascorbic acid concentration and macronutrient content.

Materials and Methods

Sample collection and preparation

Naturally grown fresh plants of *Vernonia cinerea* (VC) was collected from the Norman E. Borlaug crop research centre and other clean areas of G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India and identified by expert professional in the department of Biological Sciences. Fresh Cichorium intybus leaves without any physical damage were procured from the Council of Scientific and Industrial Research (CSIR)-Central Institute of Medicinal and Aromatic Plants (CIMAP), Pantnagar, Uttarakhand, India. The leaves of *Cichorium intybus* and the whole plant of VC Less were sorted, washed, and sanitized (soaked in 1% saline solution (NaCl) for 5 minutes to remove microbes. Herbs were further washed as per the method of with 70 % ethanol followed by twice washing with distilled water) for further processing as per the method given by (Mishra *et al.*, 2012) [22].

Drying procedure

Both the herbs were freshly cut into small pieces and subjected to two different drying methods, i.e., oven-drying and solar-drying (Mbondo *et al.*, 2018; Ukegbu and Okereke, 2013) [21, 34]. Oven drying was carried out at 45 °C in a hot air oven-drier (BT-OV6-E model; Geno Biosciences pvt. Ltd. Noida, India) with a constant air-flow rate of 2 m/s. About 500 g of fresh *V. cinerea* and *C. intybus* samples were spread on trays in a single-layer and dried to a constant weight. In case of solar drying, a small-scale solar-drier was used. Fresh *V. cinerea* and *C. intybus* samples were spread in rectangular chamber on a tray in a single-layer and dried at temperature of 35 °C ± 2 °C to a constant weight in November in Uttarakhand, India. The top part of the conventional solar drier was semicircular in shape with a radius of 50 cm and was entirely covered with a polyvinyl chloride (PVC) material. The PVC material was preferred because it filters radiations such as ultraviolet, which can destroy light-sensitive nutrients in the material being dried (Leon *et al.*, 2002) [18]. The dried plants were ground in a grinding machine (Heavy Duty Willey Mill) and sieved to give a powder with a particle size of 32 mesh (≤ 500 µm) and evaluated directly after processing.

Proximate analysis

The crude protein, crude fiber, ash, fat, and moisture content for all the prepared samples were analyzed according to the methods set forth by the Association of Analytical Chemist (AOAC, 2005) [10]. Percentage of carbohydrate content was determined by the following formula:

% Carbohydrate = 100 − (moisture % ash + % fat + % protein + % fiber content)

The physiological fuel value (Kcal/100g) of sample was calculated by the method given by (Mudambi and Rao, 1989) [24]. The energy value was calculated by summing up the products of multiplication of per cent protein, fat and carbohydrates present in the sample by 4, 9 and 4 respectively.

Physiological energy value (Kcal/100g) = (4 × protein %) + (9 × fat %) + (4 × carbohydrate %)

Ascorbic acid content analysis

According to AOAC, 2000 [1] ascorbic acid (AA) was measured by titration method using 2,6-dichlorophenol–indophenols. The content of vitamin C in dried samples was measured in mg/100 g.

Extraction procedure for phytochemical analysis

Extraction of sample was carried out according to the method described by (Bhatt and Patel, 2013) [4] with slight modification. 5 grams of dried samples (oven and solar dried) were taken in a 100ml conical flask with 15 ml of 80 percent acidified methanol and were subjected to shaking in rotatory shaker for 30 minutes at room temperature. Supernatants were decanted and the residue was re-extracted for complete extraction of phenolic and antioxidant compounds. The procedure was repeated two times and then all the supernatants were pooled and centrifuged at 6000 rpm for 15 min and filtered through Whatman No. 1 filter paper. The volume of filtrate was made up to 50 ml with 80 per cent methanol and then the sample was stored at -20 °C for further analysis.

Determination of total phenolic content (TPC)

The Total Phenol content was determined by the method

provided by (Singleton *et al.*, 1999) [33] using Folin-Ciocalteau reagent. A known aliquot of sample was taken and the volume was made up to 1.5ml with distilled water. 0.5ml of Folin-Ciocalteau reagent was added to it. 10ml of 7.5% Na_2CO_3 was also added to it and incubated at 37 °C for 60 minutes thereafter. The resulting blue color complex was read at 750 nm against blank using a UV-visible spectrophotometer. Standard series of known concentration of gallic acid (5-20 µg) was prepared. Aliquots of 0.1, 0.2, 0.3, 0.4 ml were taken and volume was made up to 5ml with distilled water and treated in the same procedure as the sample. Blank was prepared by taking 1.5ml of distilled water and treated same as sample. Results were expressed as mg GA/100g sample.

Determination of total flavonoid content (TFC)

Total flavonoid content was determined by the method given by (Zhishen *et al.*, 1999) [37]. Different sample aliquots were taken in test tubes and the volume was made upto 5 ml with distilled water and then 0.3 ml of 5% Sodium Nitrite was added to it. After 5 min, 0.6 ml of AlCl3 was added and mixed properly. Then after 6 min, 2 ml of 1 N NaOH was added to it and mixed well. 2.1 ml of distilled water was added thereafter to make the volume upto 10 ml. The absorbance of the resulting pink color complex was read at 510 nm against blank by using a UV-visible spectrophotometer. Standard series was prepared using known concentration of Rutin (50-200 µg). Aliquots of 0.5, 1.0, 1.5, 2.0 ml were prepared and volume was made up to 5ml with distilled water and treated as sample. Blank was prepared by taking 5 ml of distilled water and treated same as sample.

Statistical analysis

All treatments were carried out in triplicate. The results are reported as the mean ± standard deviation (SD). One way ANOVA was used for the statistical analysis using SPSS program (version 20 SPSS Inc., USA). The values were considered to be significantly different when $P<0.05$. Fisher's Least Significant Difference (LSD) test was applied to separate statistically significant means (at the 5 % level).

Results and Discussion

Effect of drying methods on proximate composition

Table 1: Effect of drying temperature on proximate composition (per 100g) of *Vernonia cinerea* (whole plant) *and Cichorium intybus* leaves

Nutrients (g/100g)	*V. cinerea*			*C. intybus*		
	Fresh	Solar dried	Oven dried	Fresh	Solar dried	Oven dried
Moisture (%)	86.33±1.02^a	08.73±0.27^b	10.16±0.62^c	89.00±0.70^d	08.40±0.30^e	10.50±0.70^f
Ash	0.58±0.11^a	12.65±1.00^b	10.41±0.31^c	0.53±0.23^d	10.58±0.62^e	9.68±0.94^f
Fat	0.93±0.09^a	1.06±0.11^a	1.27±1.02^a	0.66±0.09^b	0.73±0.11^b	0.80±0.04^b
Protein	1.66±0.65^a	21.46±0.80^b	19.83±0.32^c	1.33±0.32^d	17.73±0.40^e	15.63±1.06^f
Fiber	2.30±0.51^a	26.36±0.52^b	23.13±0.77^c	1.2±0.38^d	20.75±1.17^e	16.96±0.52^f
Carbohydrate	8.20±2.0^a	29.74±1.91^a	35.20±0.83^b	7.28±0.07^c	41.81±0.87^d	46.43±0.61^e
Energy value (Kcal/100g)	47.81±9.78^a	214.93±4.26^b	223.4±6.78^c	40.38±1.42^d	240.83±2.15^e	243.46±2.06^f

Values are expressed as mean ± standard deviation of triplicate determination. Means values followed by the different superscript letters along the same row are significantly different ($p<0.05$).

Values of the proximate composition (moisture, total ash, fat, protein, crude fiber, carbohydrates and energy value) for both fresh and dried *V. cinerea* and *C. intybus* are shown in Table 1. Influence of different drying techniques on the proximate composition was observed with significant differences ($p<0.05$) between the samples.

The moisture content of fresh *V. cinerea* (whole plant) and *C. intybus* (leaves) was found to be high in the range of 86.33% to 89.00% (Table 1). This was expected since most fresh leaves have very high moisture content. In a study, the moisture content of fresh *Cichorium intybus* leaves have been found in range from 91.79% - 85.53% (Jančić *et al.*, 2016) [14]. Moisture content reduced significantly in solar dried *V. cinerea and C. intybus* (08.73±0.27% and 08.40±0.30%) when compared to oven dried (10.16±0.62% and 10.50±0.70%), respectively. These observations agree with (Cheptoo *et al.*, 2019) [6]. This may be attributed to structural changes in the plant material that lose more moisture content via transpiration and hydrolysis of stored starch content. The moisture contents of all dried samples obtained from the two drying methods were below 15% which is a critical factor in preservation of herbs (Orph Anides *et al.*, 2013) [28].

In this study, the ash content of the solar dried *V. cinerea* and *C. intybus* was significantly higher ($P<0.05$) in the range of 10.58 to 12.65 g/100g as compared to oven dried and fresh plant material. The higher ash value of solar dried samples is similar to another report (Onwuka *et al.*, 2002) [27]. High ash content in solar dried and oven dried samples observed in this study could be due to the removal of moisture, which tends to increase the concentration of nutrients (Cheptoo *et al.*, 2019) [6].

The fiber content was significantly higher (26.36% and 20.75%) in the solar-dried samples of *V. cinerea* (whole plant) and *C. intybus* (leaves), respectively as compared to oven- dried and fresh samples as shown in table 1. The higher fibre values for the dried herbs were due to loss of moisture and plants are good sources of fibre. In addition, it is known that the loss of moisture increases nutrient density in foods of which fibre is one.

The lipid content was numerically but not significantly high in oven dried *V. cinerea* and *C. intybus* (01.27g/100 and 00.80g/100) followed by solar drying and fresh plant materials. The body does not manufacture essential fatty acids and diet like bitter leaf may supply these (Morris *et al.*, 2004; Adeyeye and Okotiti, 1999) [23, 2]. All the present observations (moisture, ash, fat and fiber) agreed with a study of another plant, *Vernonia amygdalina* (bitter leaf) that belongs to same genus (Garba and Oviosa, 2019) [9].The ash and fiber content of solar dried *Vernonia amygdalina* were enhanced as compared to oven dried samples and it ranged from 10.92 ± 0.03g/100g to 11.15 ± 0.04g/100g and 3.54 ± 0.06g/100g to 3.63 ± 0.03g/100g, respectively. Moisture and fat content of solar dried sample were decreased from 13.07 ± 0.02g/100g to 12.80 ± 0.01g/100g and

2.53 ± 0.21g/100g to 2.49 ± 0.17g/100g, respectively.

The result obtained from the present research work also revealed that the protein content was significantly higher (21.46g/100g and 17.73g/100g) in the solar-dried sample of *V. cinerea* whole plant and *C. intybus* leaves, respectively followed by oven-dried and fresh herbs. This is in agreement with (Kirruti *et al.*, 2021) [16]. The change in protein content could be attributed to mild heating effect associated with all the drying conditions which could result in the unzipping of hydrophobic forces leading to a partial distribution of the primary, secondary, tertiary and quaternary structure of the protein molecule (Ihekoronye and Ngoddy, 1985) [12].

The carbohydrate content of both (*V. cinerea* and *C. intybus*) oven dried plants was in the range of 35.41g/100g to 46.70g/100g, respectively followed solar dried and fresh plants. It was observed that all the values were significantly different from each other. The energy value of the oven dried samples was observed significantly higher. (P<0.05) (Table1). The increase in these nutrients could be attributed to the loss of moisture due to drying.

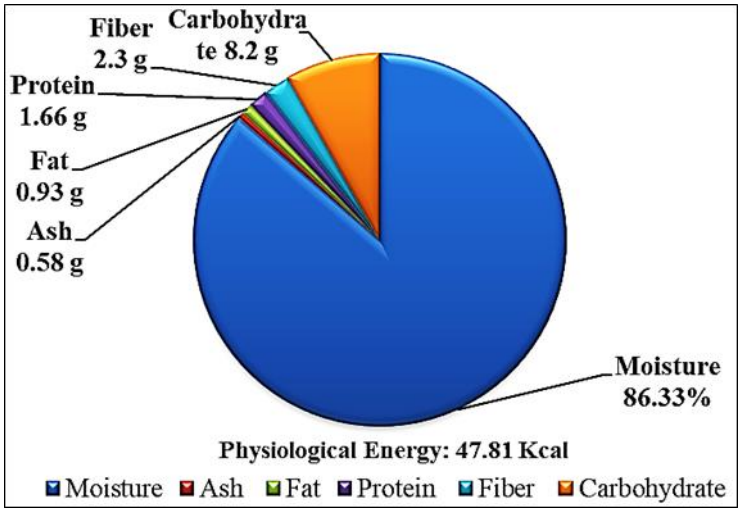

Fig 1: Proximate composition of fresh whole plant of *Vernonia cinerea*

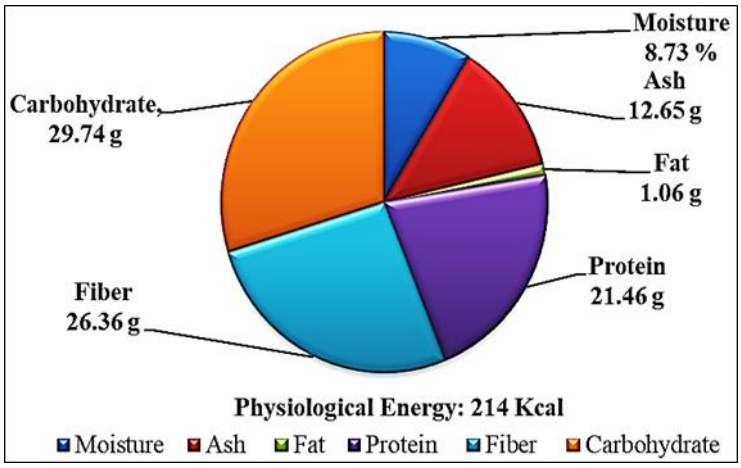

Fig 2: Proximate composition of solar dried whole plant of *Vernonia cinerea*

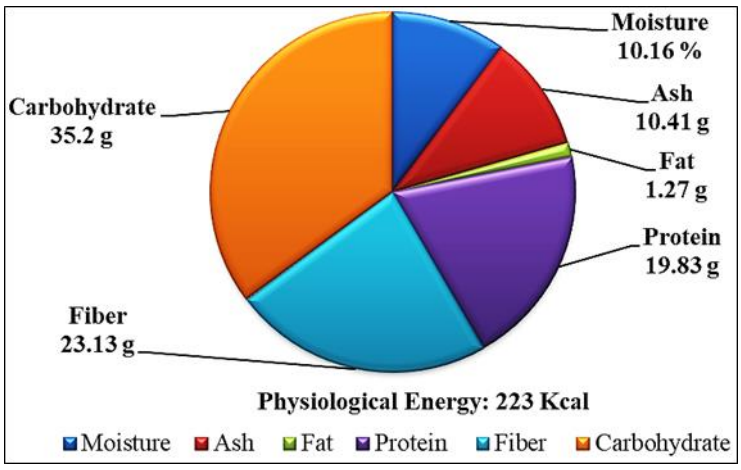

Fig 3: Proximate composition of oven dried whole plant of *Vernonia cinerea*

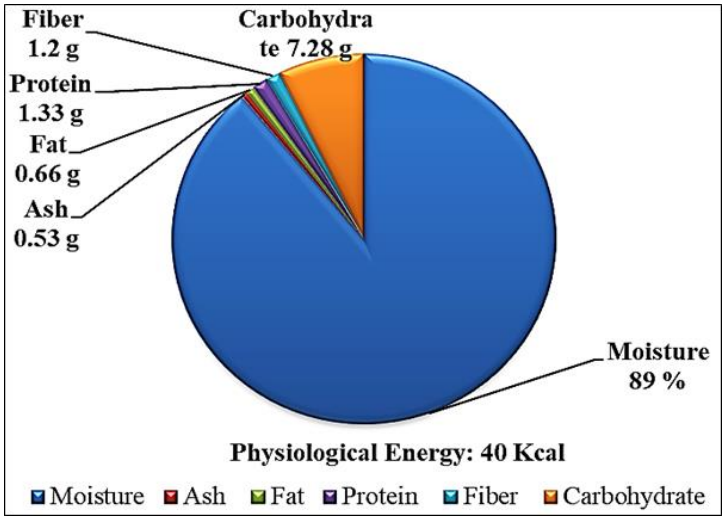

Fig 4: Proximate composition of fresh leaves of *Cichorium intybus*

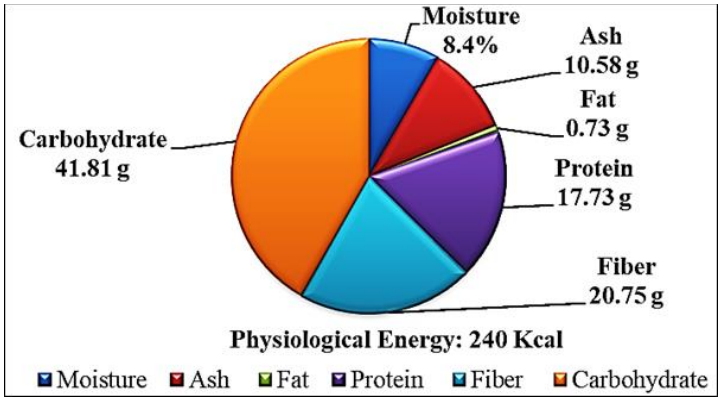

Fig 5: Proximate composition of solar dried leaves of *Cichorium intybus*

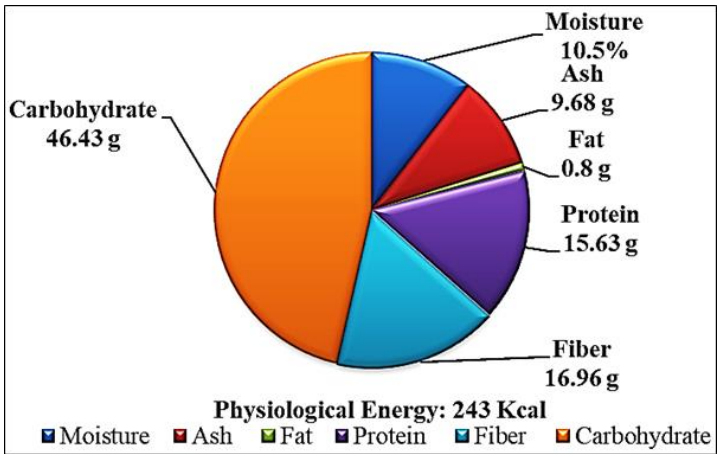

Fig 6: Proximate composition of oven dried leaves of *Cichorium intybus*

On the basis of observations presented in table1, there is a significant effect of both the drying methods viz. solar drying and oven drying on the moisture, ash, protein, fiber and carbohydrate content of *V. cinerea* and *C. intybus*. The data also reveals that retention of macro-nutrients is better by solar drying technique.

Effect of drying temperature on ascorbic acid

Table 2: Effect of drying temperature on ascorbic acid (mg /100g) of *Vernonia cinerea* (whole plant) *and Cichorium intybus* (leaves)

Ascorbic acid (mg/100g)	*Vernonia cinerea*	*Cichorium intybus*
Fresh	129.16±2.94[a]	41.66±1.94[d]
Solar dried	102.08±2.94[b]	35.41±2.23[e]
Oven dried	52.08±2.89[c]	22.91±2.09[f]

Values are expressed as mean ± standard deviation of triplicate determination. Means values followed by the different superscript letters along the same column are significantly different (p<0.05)

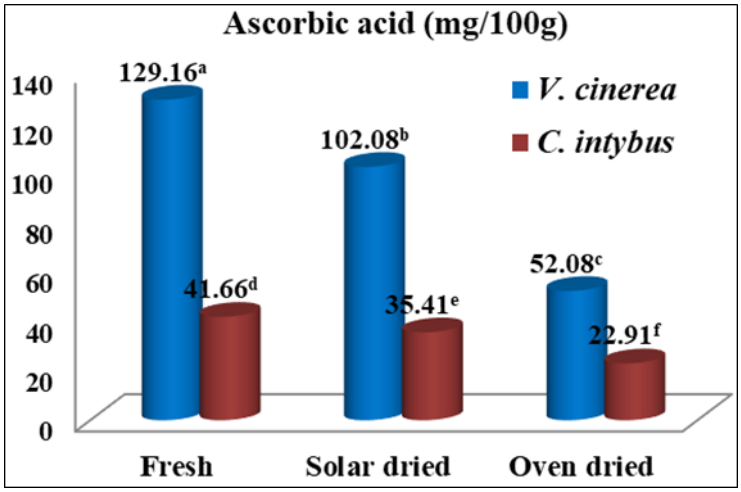

Fig 7: Values are expressed as mean of triplicate determination. Mean values followed by the different letters on the each bar are significantly different (p<0.05)

The quantities of vitamin C in the fresh and dried *V. cinerea* and *C. intybus* are shown in fig.1, with a substantial (p<0.05) reduction during both the drying process. When dried in solar drier (35±2 °C) and hot air oven (45 °C), the concentration of vitamin C in *Vernonia cinerea* and *Cichorium intybus* was reduced from 102.08 mg/100 g to 52.08 mg/100 g and 25.41 mg/100 g to 22.91 mg/100 g, respectively as compared to fresh samples (129.16 mg/100g and 41.66 mg/100g, respectively). Okmen and Bayindirli, 1999 [26], reveals that thermal processing of vitamin C demonstrated the loss of ascorbic acid in a greater amount than traditional drying procedures. Therefore, the content of vitamin C found in the dried herbs decreased as drying temperature increased, although it still remained relatively moderate in amount, which may be considered a valuable asset for preparation of herbal Ready-to-Eat food products or herbal infusion in tea mixtures, etc. On the other hand, the presence of vitamin C in the dried samples may be used as a quality index and also as an evaluating criterion for thermal treatment.

Effect of drying temperature on Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Table 3: Effect of drying temperature on TPC (mg GAE/100g) and TFC (mg RE/100g) of *Vernonia cinerea* (whole plant) and *Cichorium intybus* (leaves)

Bioactive compounds	*V. cinerea*			*C. intybus*		
	Fresh	Solar dried	Oven dried	Fresh	Solar dried	Oven dried
Total Phenolic Content	1666.66±2.51^{a}	2945±1.41^{b}	2261.66±1.69^{c}	1946.33±1.24^{d}	3602.33±1.69^{e}	3030.66±0.94^{f}
Total Flavonoid Content	420.66±2.49^{a}	923±0.81^{b}	853.66±2.62^{c}	265.33±2.49^{d}	673.66±2.86^{e}	553.66±2.62^{f}

Values are expressed as mean ± standard deviation of triplicate determination. Means values followed by the different superscript letters along the same rows are significantly different (p<0.05)

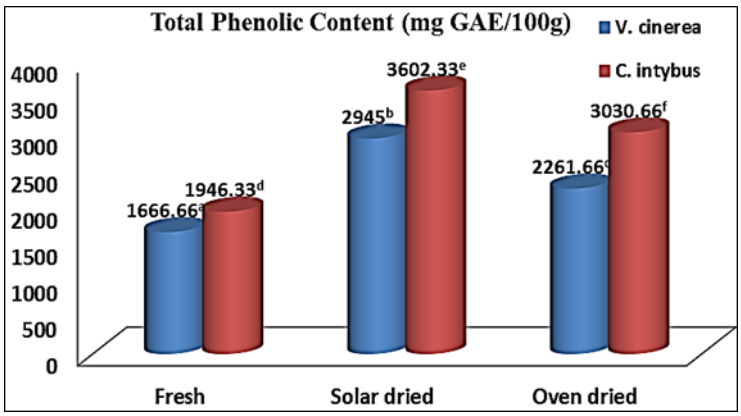

Fig 8: Effect of drying temperature on total phenolic content of *Vernonia cinerea* and *Cichorium intybus*. Identical uppercase letters above the bars indicate significant differences (p<0.05) in total phenolics

Total phenolic content was found to be higher in solar dried *V. cinerea* and *C. intybus* (2945 mg GAE/100g and 3602.33 mg GAE/100g), respectively followed by oven dried samples (2261.66 mg GAE/100g and 3030.66 mg GAE/100g) and fresh samples (1666.66 mg GAE/100g and 1946.33 mg GAE/100g) respectively. Fig. 2 shows the comparative differences in total phenolics for the solar dried, oven dried and fresh herbs. Significant differences (p<0.05) in total phenolic content were observed in the present research.

The higher total phenol content of solar-dried as compared to

oven dried and fresh plants could be linked to more efficient extraction of the insoluble phenolic compounds such as condensed tannins, and phenolic acids (Farag *et al.*, 2013; Komes and Dra 2013; Roshanak *et al.*, 2016) [8, 17, 32] bound to cell wall polysaccharides or proteins (Giada and Maria 2016) [10]. During drying processes phenolic-sugar glycosidic bonds may be cleaved with heat treatment leading to the formation of phenolic aglycons, which react better with the Folin-Ciocalteu reagent leading to higher values of total phenolics (Singleton, 1999) [33]. The variation in phenolic content between the oven-dried and solar-dried herbs might be attributed to the different drying temperatures used. It has been reported that the phenolic content of solar-dried and oven-dried chia leaves improved dramatically (Kirruti *et al.*, 2021) [16]. The preservation of phenolic content can be due to drying heat permeating and dissolving the membrane's leaves, releasing complex phenolics. This finding is in line with the findings of (Kirakou *et al.*, 2017) [15], which discovered improved phenolic content in solar dried cowpea leaves. When compared to solar-dried leaves, the TPC of oven-dried herbs was lower. Thermal breakdown of phenolic compounds at extreme temperatures, such as those encountered in the oven drying procedure, might explain the reduction in phenolic concentration in oven-dried samples. These findings are consistent with information given by (Mbondo *et al.*, 2018) [21], where a reduction was found in phenolic acid after oven drying eggplant leaves at 60 °C.

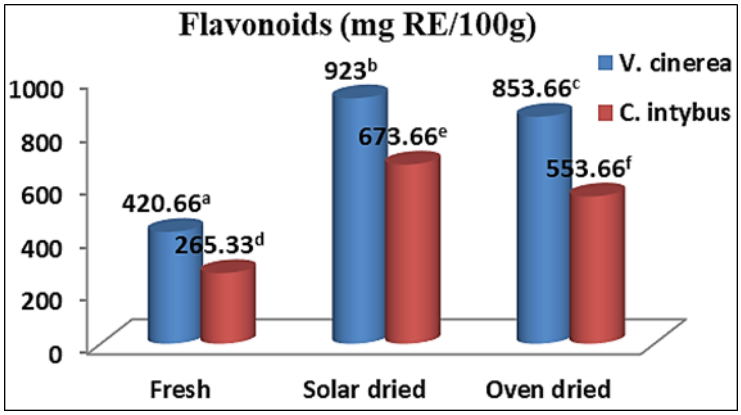

Fig 9: Effect of drying temperature on total flavonoid content of Vernonia cinerea and Cichorium intybus. Identical uppercase letters above the bars indicate significant differences (p<0.05) in total flavonoids

The total flavonoid content of solar dried *Vernonia cinerea* and *Cichorium intybus* was in the range of 923 mg RE/100g to 673.66 mg RE/100g, respectively, which fell dramatically to 853.66 mg RE/100g and 553.66 mg RE/100g following oven drying and 420.66 mg RE/100g and 265.33 mg RE/100g in fresh samples, respectively (fig.3). When compared to oven dried herbs, solar dried herbs showed significantly higher flavonoid content. Flavonoids are heat sensitive, and excessive heat exposure causes them to degrade. The fact that solar drying has a lower heat coefficient than oven drying, which has a higher heat convective transfer, could be attributed to the high flavonoid retention in solar-dried samples. These findings are in line with the findings of (Kirruti *et al.*, 2021) [16].

Conclusion

The composition of herbal material might vary due to different plant locations or maturity. In this study, we analyzed how two different drying techniques viz. solar drying and oven drying influenced the proximate principles, ascorbic acid content, total phenols and total flavonoids composition of dry *V. cinerea* and *C. intybus* herbs. Our study concluded that solar drying was a more efficient method in reducing moisture to preserve the nutrients like crude protein, crude fiber, ash and carbohydrates, as compared to the oven drying technique. Both the herbs retained more total phenol, total flavonoids and ascorbic acid using solar drying technique. The statistical analysis showed that the nutritional and bioactive composition of the solar dried and oven dried plants varied significantly. Drying is one of the most popular strategies for preservation of seasonal and perishable greens. The abundantly available inexpensive herbs; *V. cinerea* and *C. intybus* that are a pool house of nutrients can be used in the developing countries to address metabolic diseases. The findings of the study are of relevance for the food and pharmaceutical industries as compositional stability and reproducibility are required for the further nutraceutical use of the herbs. While the current study demonstrates that plant materials are viable alternative food sources, more in depth research is required for further refining the drying techniques.

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