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EFFECT OF DIFFERENT DRYING METHODS ON NUTRITIONAL COMPOSITION, ANTIOXIDANT ACTIVITY AND PHYTOCHEMICALS OF *Enhydra fluctuans*

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ABSTRACT

Enhydra fluctuans is a common edible plant, showcases diverse biological advantages. This research investigates the effects of various drying methods (sun, oven, cabinet, vacuum, and freeze) on nutritional, antioxidant, and phytochemical attributes. By comparing outcomes with fresh leaves, we utilized five drying techniques, proximate composition, antioxidant activity, and phytochemical content (TFC, TPC). Findings reveal lowered level of ash and moisture, alongside elevated carbohydrate, fat, fiber, protein, antioxidant activity, Total Flavonoid Content (TFC), and Total Phenolic Content (TPC). While oven drying produces high levels of ash, fat, and fiber, sun drying records the highest moisture and lowest TFC. Vacuum drying yields lowest ash, fat, antioxidant activity and TPC. Freeze drying boasts highest protein (17.50±0.35%), carbohydrate (55.87±0.18%), antioxidant activity (488.21±1.25%), TPC (0.56±0.13mgQAE/g), and lowest fiber, moisture. Cabinet drying presents least carbohydrate. Oven drying has maximum energy (335.16±0.18 Kcal/100g), vacuum drying minimum. Statistically, moisture, protein, fiber, total energy, TFC, TPC, antioxidant activity are significant ($p<0.05$). However, dried sample's carbohydrate, ash, and fat content are statistically insignificant ($p>0.05$). In conclusion, among five dried samples, oven and freeze-dried exhibit notable significance as per the study's outcomes.

1. Introduction

Nature has provided medicinal substances for thousands of years, and numerous modern pharmaceuticals have been obtained from natural sources, some of which have historical evidence for their usage in traditional medicine. *Enhydra fluctuans*, a tropical herb, commonly known as helencha or harkuch, belonging to family Asteraceae, is gaining lot of importance for its therapeutic potentials (Rahman, 2015). This is an edible semi-aquatic herbaceous vegetable plant with serrate leaves, grows commonly all over the country. Common names for *Enhydra fluctuans* include "water crest" and "marsh herb." It is a separate-leaved, annual vegetable plant that is edible, semi-aquatic, and nonwoody. Tropical and subtropical areas are home to *Enhydra fluctuans* (Ali *et al.,* 2013). This plant is mostly found in Assam and the North-Eastern part of India (Chakraborty *et al.,* 2012). It is a widely consumed vegetable in Bangladesh and a nutrient-rich source of proteins, carbs, vitamins, and minerals (Sattar *et al.,* 2016).

In Bangladesh, the plant has also been used as a traditional medicine in addition to being used as food. Flavonoids, alkaloids, saponins, tannins, phenols, beta-carotene, protein, and carbohydrates are among the phytochemicals found in this medicinal plant (Sarma *et al.,* 2014;

Jayashree, 2013; Dewanji *et al.,* 1993; Hazra *et al.,* 2012; Satyajit, 2012; Kuri *et al.,* 2014). At least 35 distinct chemicals, mostly from the phytochemical groups of flavonoids, isoflavonoids, steroids, and terpenoids, have been found in *E. fluctuans*. According to (Barua *et al.,* 2021) the total phenolic content was found to be 60.67±0.083 g/ml GAE and 39.83±0.083 g/ml GAE for the ethanolic and aqueous extract, respectively. According to (Ghosh *et al.,* 2007), the plant's aerial portions have substantial antibacterial and anthelmintic properties. From this plant, terpenes (Krishnaswamy *et al.,* 1995), sesquiterpene lactones (Ali *et al.,* 1972), and carotene have all been recognized as chemical components. According to (Alfasane *et al.,* 2018), raw *Enhydra fluctuans* had 317.28 (kcal/100g) of energy, 14.00% ash, 18.20% protein, 1.14% fat, 11.50% fiber, and 56.60% carbohydrate.

Recently, the ability of crude extract and various fractions to scavenge free radicals was observed (Sannigrahi *et al.,* 2010). It has been noted that the leaves of E. fluctuans have hypotensive properties (Joshi and Kamat, 1972). It has many beneficial effects such as antioxidant activity (Uddin *et al.,* 2005); anti-cancer activity (Kumar *et al.,* 2012); antidiarrhoeal activity (Kumar and Khanum, 2012); hepatoprotective activity (Patil *et al.,* 2008); analgesic activity; neuroprotective potential (Alebiosu *et al.,* 2015); antidiabetic (Khan and Yadava 2010), anthelmintic and thrombolytic (Kuri *et al.,* 2014), Phagocytic and cytotoxic activity (Hassan *et al.,* 2015).

Drying is the process of removing extra water while preserving nutritional content, enhancing visibility, and preparing an item for usage. There are various methods of drying, including sun, vacuum, freeze, oven, and cabinet drying. Raw and dried samples have different nutritional compositions, antioxidant activities, and phytochemical components. A dried sample may have a lot or little in the way of nutritious components. At present the powder form of *Enhydra fluctuans* used as a drug for medicinal purposes that why it's necessary to know the effects of different drying on *E. fluctuans*. This study aims to investigate the impacts of various drying techniques on proximate composition, evaluating the antioxidant activity, determining the total phenol and total flavonoid content of dried *Enhydra fluctuans*. Therefore, our research on the antioxidant activity and functional potential of this plant will be helpful in both choosing plants as natural alternatives to medications for dietary supplements and in the development of antioxidant-based medications.

2. Materials and methods

The experiment was conducted in the department of Food Technology and Nutritional Science, Mawlana Bhashani Science and Technology University, Tangail. The study was carried out from January 2023 to March 2023.

2.1. Collection and preparation of sample

In this study, Enhydra fluctuans were collected from the local area of Tangail, Bangladesh. The foreign objects, rotten materials were removed from the collected sample and washed using lukewarm water to eliminate dirt and chemical stains. After washing, a brief period of rest was allowed for water drainage. The sample was then cut into appropriate sizes based on the chosen drying method.

2.2. Drying methods

The study employed five distinct drying methods. In sun drying, about 500 g samples were cleaned and chopped. These were laid in single layers on racks, rotated for uniform evaporation, and kept indoors at night. After around two days of drying, the samples cooled indoors before grinding. In vacuum drying, 400 g of samples were cut into pieces measuring 1 to 1.5 inches in length. Each tray of the vacuum dryer was loaded with a 100 g sample, spread thinly, and subjected to a temperature of 70°C. After 10 hours, the samples were removed from the vacuum dryer and left to cool. Following the cooling process, the samples were prepared for grinding. In terms of oven drying, a clean sample weighing 300 grams was obtained and subsequently divided into medium-sized pieces.

These fragments were then evenly distributed across the trays within a microwave dryer. After five hours, the sample was carefully extracted from the oven dryer. To ensure proper cooling, the sample was allowed to rest briefly before it could be subjected to the grinding process. For the freeze drying process, *Enhydra fluctuans* weighing 260 grams was meticulously divided into uniformly small pieces. These segments were then placed within a freeze dryer, operating at an approximate temperature of -68°C. Following a period of 7 to 8 hours, the sample was retrieved from the freeze dryer. After a short resting period, the sample was prepared for the subsequent grinding process. In the cabinet drying methods, a total of 300 grams of sample material was segmented into medium-sized pieces. These pieces were uniformly distributed among the trays within the cabinet drier. Upon completion of a one-day drying period, the sample was carefully removed from the cabinet drier.

Subsequent to each drying method, all the dried samples were subjected to grinding process using an electric grinder. The resulting powders were meticulously stored within separate plastic jars, each with a secure seal, in order to safeguard them against moisture absorption.

2.3. Proximate analysis of raw and dried *Enhydra Fluctuans*

The moisture, ash, carbohydrates, crude fat were determined by (AOAC, 2000) method. Protein content was determined by Kjeldahl method (Bradstreet, 1954). Crude fiber was determined by (AOAC, 1995).

2.4. Determination of phytochemical content

Total phenolic content (TPC) was determined by Folin-Ciacalteu method (Premathilaka, 2016) and Total flavonoid content (TFC) was determined by aluminum chloride colorimetric test (Kamtekar *et al.,* 2014). In the case of TPC, a Gallic acid curve was established using different dilutions (0.1, 0.01, 0.001, 0.0001, 0.00001 mg/ml) in methanol. Each dilution (100µl) mixed with water (500µl) and Folin-Ciocalteu reagent (100µl), stood for 6 min. Then, 7% sodium carbonate (1ml) and water (500µl) were added. Absorbance was measured at 760 nm after 90 min. The same was done with water extracts of three formulations.

Total phenolic content was calculated as mgGAE/g. All tests were triplicated. In the case of TFC, a quercetin calibration curve was established using dilutions (0.1, 0.5, 1.0, 2.5, and 5 mg/ml) from a standard 2 quercetin solution in methanol. For each dilution, 100 µl was mixed with 500 µl distilled water, then with 100 µl 5% Sodium nitrate, and stood for 6 minutes. Subsequently, 150 µl of 10% aluminum chloride solution was added and left for 5 minutes, followed by sequential addition of 200 µl 1M Sodium hydroxide solution. The mixture's absorbance was measured at 510 nm using a UV spectrophotometer. The same process was applied to all samples. Total flavonoid content was determined as mgQE/g. All steps were conducted in triplicate.

2.5. Determination of antioxidant activity

Antioxidant activity was determined by DPPH method (Brand-Williams *et al.,* 1995). To make the sample extract, 1g of the sample was put into 10ml of methanol in a beaker. It was stirred using a magnetic stirrer for 10-15 minutes and then filtered with filter paper. A new solution of DPPH (0.002%) was made in methanol and its absorbance was measured at 515 nm. Then, 50µl of the sample extract was mixed with 3ml of DPPH solution and left in the dark for 15 minutes. The absorbance was measured again at 515 nm.

% of inhibition = (Absorbance of DPPH-Absorbance of sample)/(Absorbance of DPPH)×100

2.6. Statistical analysis

The data were analyzed to know the mean and SD value and their statistical significance. SPSS Statistic software, version20.0 (SPSS Inc., Chicago, USA), was used for the statistical

treatment of the data. Analyses of variance (ANOVA) were carried out to evaluate whether there were significant differences ($p< 0.05$) amongst the samples.

3. Results and discussions

Table 1 provides a comprehensive overview of the basic nutritional components and energy content of raw *Enhydra fluctuans.* It demonstrates that raw *Enhydra fluctuans* had the following composition: 72.45± 0.69% moisture, 13.96±0.24% ash, 12.37±1.13% protein, 1.59±0.34%fat, 16.42±0.75% fiber, 54.98±4.55% carbohydrates, and 299.94±16.75 (kcal/100g) energy.

As per the findings of Datta *et al.,* (2019), the values for raw *Enhydra fluctuans* were as follows: 67.69±0.78% moisture, 15.15±0.44% ash, 8.00±0.06% protein, 1.10±0.01% fat, 15.37±0.21% fiber, 9.64±0.06% carbohydrates, and 80.53±0.16 (kcal/100g) of energy. Another study stated, raw Enhydra fluctuans contained 317.28(kcal/100g) of energy, 14.00% ash, 18.20% protein, 1.14% fat, 11.50% fiber, and 56.60% carbohydrates (Alfasane *et al.,* 2018). It became apparent that the findings of this study and those of other studies are nearly identical. But protein, carbohydrate, and calorie contents were slightly low; fiber content was high in comparison to other studies. The variations may result from the location of the crop's cultivation, the sampling techniques employed, the impact of the harvest season, etc.

Table 1. Proximate analysis of raw *Enhydra fluctuans*

Biochemical composition	Mean ± SD
Moisture (%)	72.45 ± 0.69
Ash (%)	13.96 ± .24
Protein (%)	12.37 ± 1.13
Fat (%)	1.59 ± .34
Fiber (%)	16.42 ± .75
Carbohydrates (%)	54.98 ± 4.55
Energy (Kcal/100g)	299.94 ± 16.75

Notes: Each value in the table was obtained by calculating the average of triplicate experiments (n=3) and data are presented as Mean ± Standard Deviation.

Table 2 presents the nutrient content and overall energy values of *Enhydra fluctuans* subsequent to the implementation of different drying techniques. It depicts that the sun dried sample had a moisture content of 10.81±0.19%, freeze dried sample had 3.82±0.26%, cabinet dried had 5.74±0.21%, vacuum dried had 8.22±0.10%, and the oven dried sample had 6.28±0.16%. According to this investigation, the sun dried sample had the highest moisture content, whereas the freeze dried sample had the lowest. Here, the moisture content of dried samples among five different drying were statistically significant ($p<0.05$). The amount of ash produced by Enhydra fluctuans dried using various drying methods were represented (Table 2). It depicts that the ash content were 13.38±1.79%, 13.05±0.93%, 13.62±1.18%, 12.34±0.58% and 13.63 ±1.01% for the sun, freeze, cabinet, vacuum and oven dried sample respectively. Though the differences among the values are not so notable, oven dried samples had the highest ash content of all the dried samples and vacuum dried had less. There is no significant significance ($p > 0.05$) were observed among them.

Table 2.Nutrients content and total energy of *Enhydra fluctuans,* after the application of various drying methods

Nutritional parameters	Drying techniques					P value
	Sun Mean ± SD	Cabinet Mean ± SD	Vacuum Mean ± SD	Oven Mean ± SD	Freeze Mean ± SD	
Moisture (%)	10.81±0.19	5.74±0.21	8.22±0.10	6.28±0.16	3.82±0.26	0.000
Ash (%)	13.38± 1.79	13.62±1.18	12.34± 0.58	13.6 ± 1.01	13.05±0.93	0.651
Carbohydrate (%)	55.61±0.49	55.53±0.56	55.64±0.27	55.57±0.75	55.87±0.18	0.923
Protein (%)	14.08± 0.28	15.36± 0.44	14.64± 0.13	13.23± 0.23	17.50± 0.35	0.000
Fat (%)	4.88 ± 0.73	4.04 ± 1.82	3.52 ± 1.21	6.20 ± 0.94	3.99 ± 0.41	0.100
Fiber (%)	20.38±0.77	16.06±2.50	21.02±2.46	23.86±2.95	7.55±1.21	0.000
Total energy (kcal/100g)	322.70±0.16	319.61±0.28	312.52±0.39	335.16±0.18	329.65±0.25	0.000

Notes: Each value in the table was obtained by calculating the average of triplicate experiments (n=3) and data are presented as Mean ± Standard Deviation. Statistical analysis were carried out by Turkeys test at 95% confidence level and statistical significance were accepted at the $p < 0.05$ level.

Table 3.Phytochemical composition of *Enhydra fluctuans*, following various drying techniques

Phyto-chemicals	**Raw and dried samples**						P value
	Raw Mean ± SD	Sun Mean ± SD	Cabinet Mean ± SD	Vacuum Mean ± SD	Oven Mean ± SD	Freeze Mean ± SD	
TFC (mgQE/g)	8.05±0.63	12.86±0.85	16.20±0.39	12.94±1.18	16.31±0.71	13.90±0.10	0.000
TPC (mgQAE/g)	0.15±0.02	0.37±0.03	0.55±0.09	0.28±0.36	0.46±0.05	0.56±0.13	0.005

Notes: Each value in the table was obtained by calculating the average of triplicate experiments (n=3) and data are presented as Mean ± Standard Deviation. Statistical analysis were carried out by Turkeys test at 95% confidence level and statistical significance were accepted at the $p < 0.05$ level. TFC =Total flavonoid content; TPC= Total Phenolic Content.

Table 4. Measured antioxidant activity of *Enhydra fluctuans* after the application of various drying methods

Biochemical parameter (%)	**Samples**	**Mean±SD**	**P value**
Antioxidant activity	Raw	19.91±1.67	0.000
	Sun	480.60±1.66	
	Freeze	488.21±1.25	
	Vacuum	372.86±5.77	
	Cabinet	480.60±1.87	
	Oven	481.77±1.05	

Notes: Each value in the table was obtained by calculating the average of triplicate experiments (n=3) and data are presented as Mean ± Standard Deviation. Statistical analysis were carried out by Turkeys test at 95% confidence level and statistical significance were accepted at the $p < 0.05$ level.

Table 2 presents the nutrient content and overall energy values of *Enhydra fluctuans* subsequent to the implementation of different drying techniques. It depicts that the sun dried sample had a moisture content of 10.81±0.19%, freeze dried sample had 3.82±0.26%, cabinet dried had 5.74±0.21%, vacuum dried had 8.22±0.10%, and the oven dried sample had 6.28±0.16%.According to this investigation, the sun dried sample had the highest moisture content, whereas the freeze dried sample had the lowest. Here, the moisture content of dried samples among five different drying were statistically significant ($p<0.05$). The amount of ash produced by *Enhydra fluctuans* dried using various drying methods were represented (Table 2). It depicts that the ash content were 13.38±1.79%, 13.05±0.93%, 13.62±1.18%, 12.34±0.58% and 13.63 ±1.01% for the sun, freeze, cabinet, vacuum and oven dried sample respectively. Though the differences among the values are not so notable, oven dried samples had the highest ash content of all the dried samples and vacuum dried had less. There is no significant significance ($p > 0.05$) were observed among them.

In the context of carbohydrate contents, the results showed the carbohydrate content of the sun, freeze, cabinet, vacuum and oven dried samples were statistically insignificant ($p>0.05$) which were accounted for 55.61±0.49%, 55.87±0.18%, 55.53±0.56%, 55.64±0.27% and 55.57 ±0.75% in the specified sequence. The sample that was freeze dried had the most carbohydrate (55.87±0.18%), whereas the sample that was cabinet dried had the least (55.53±0.56%), according to this experiment (Table 2). Furthermore, the protein concentration in dried *Enhydra fluctuans* obtained through various drying techniques exhibits notable variation. In this study, the protein content in the freeze-dried sample was higher (17.50± 0.35%) and lower (13.23 ± 0.23%) in the oven-dried sample. The protein content of all dried samples were statistically significant ($p<0.05$).

Table 2 displayed the variation in fat content among dried *Enhydra fluctuans* samples subjected to diverse drying techniques. The findings of this investigation highlight notable distinctions in fat content depending on the drying technique employed. Notably, the oven-dried samples exhibited the highest fat content (6.20±0.94%), while the vacuum-dried samples demonstrated the lowest fat content (3.52±1.21%) among the various techniques examined. In this instance, the fat content of various dried *Enhydra fluctuans* samples was not statistically significant ($p>0.05$).

The results from the table 2 shows varying fiber contents: sun-dried (20.38±0.77%), freeze-dried (7.55±1.21%), cabinet-dried (16.06±2.50%), vacuum-dried (21.02±2.46%), and oven-dried (23.86±2.95%). Notably, oven-dried samples had the highest fiber content (23.86±2.95%), while freeze-dried samples had the lowest (7.55±1.21%). Here, the fiber content of various dried *Enhydra fluctuans* sample has a p value of 0.00, which is less than 0.05. Therefore, this value is significant.

It was discovered that the oven-dried sample had the highest energy content (335.16±0.18 Kcal/100g) and the vacuum-dried sample had the lowest (312.52±0.39 Kcal/100g) (Table 2). Total energy of all dried samples were statistically significant ($p<0.05$).

Table 3 lists the total flavonoid content of raw and various dried *Enhydra fluctuans*. It exhibits that the total flavonoid content of the raw sample was 8.05±0.63 mgQE/g. The sun dried sample contained 12.86±0.85 mgQE/g, freeze 13.90±0.10 mgQE/g, cabinet 16.20±0.39 mgQE/g, vacuum 12.94±1.18 mgQE/g and oven 16.31±0.71QE mgQE/g of TFC. It stated that the raw sample's total flavonoid content was lower than that of the dried samples. Furthermore, among the dried samples, TFC was higher in the oven-dried sample (16.31±0.71mgQE/g) and sun dried contained less (12.86±0.85mgQE/g). The total flavonoid content of all dried samples were statistically significant ($p<0.05$). These significant variations may result from different genotypes or different location as indicated by Alam *et al.*, (2015). Total Phenolic Content (TPC) of raw and different dried *Enhydra fluctuans* was shown in Table 3.To start with,

total phenolic content of the raw sample was 0.15±0.02 mgQAE/g. Besides, the TPC of sun, freeze, cabinet, vacuum and oven dried sample were 0.37±0.03 mgQAE/g, 0.56±0.13 mgQAE/g, 0.55±0.09 mgQAE/g, 0.28±0.36 mgQAE/g, and 0.46±0.05 mgQAE/g respectively. It showed that the raw sample's total phenol level was lower than that of the dried samples. Additionally, among the dried samples, TPC was higher in the freeze dried sample (0.56±0.13) and vacuum contained less (0.28±0.36). In this case, the total phenolic content among five distinct drying samples were statistically significant ($p<0.05$).

Table 4 presents the antioxidant activity of raw and various dried forms of *Enhydra fluctuans*. The raw sample exhibited an antioxidant activity of 19.91±1.67%. Among the dried samples, sun-dried showed an antioxidant activity of 480.60±1.66%, freeze-dried had 488.21±1.25%, cabinet-dried resulted in 480.60±1.87%, vacuum-dried yielded 372.86±5.77%, and oven-dried displayed 481.77±1.05% antioxidant activity. It was observed that the raw sample's antioxidant activity was lower compared to the dried samples. Furthermore, within the dried samples, freeze-dried samples exhibited the highest antioxidant activity (488.21±1.25%), while vacuum-dried samples had comparatively lower activity (372.86±5.77%).

4. Conclusion

In this study, ash, fat, fiber, TFC contents were in the highest amount and moisture, protein content were in the lowest amount in oven dried sample. In vacuum drying, moisture content was the highest amount while ash, fat, TPC contents were the lowest. In freeze drying, protein, carbohydrate, and TPC were in the highest amount and fiber contents were in the lowest amount. TFC and carbohydrate contents were in the lowest amount in sun and cabinet dried sample respectively. Total energy was the highest in oven dried sample and the lowest in vacuum dried sample. This study outcome enables us to draw the conclusion that in comparison to five different dried samples the oven dried sample demonstrated the significant characterization.

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