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EFFECT OF DRYING METHODS ON PROXIMATE COMPOSITION AND PHYTOCHEMICAL CONTENT OF SESBANIA GRANDIFLORA LEAVES

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Abstract:

The leaves of *Sesbania grandiflora* (L.) Poir, family Fabaceae, is a rich source of nutritional and phytochemical properties. This study was conducted in Yezin Agricultural University, Nay Pyi Taw, Myanmar. The treatments were fresh, shade and oven drying. The aim of the research was to investigate the influence of two drying methods (shade drying and oven drying) on proximate composition and phytochemical content of *S. grandiflora* (L.) Poir leaves. The results showed that shade-dried samples had the highest crude protein, crude ash, and carbohydrate while oven-dried samples had the highest crude fiber and crude fat content. Alkaloids, flavonoids, tannins, saponins, and terpenoids were found in the sample in all treatments. Antioxidant activity and vitamin C content were the highest in fresh samples and total phenol content was the highest in shade-dried samples. Thus, *S. grandiflora* is a good source of phytochemical and could be utilised for health promoting food item.

Key Words: Shade drying, oven drying, proximate composition, phytochemical, antioxidant

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1. Introduction

Sesbania grandiflora L. belongs to the family Fabaceae. It is native to India, Indonesia, Malaysia, Myanmar, and Philippines. It contains rich source of vitamin C and antioxidant (Mohiuddin, 2019). It is also a useful dietary supplement to control postprandial blood glucose as a potential remedy for controlling type 2 diabetes mellitus (Thissera et al., 2020).

Drying is a process to remove moisture, reduce water, and minimize deteriorative biochemical reactions (Buchaillot et al., 2009). Moreover, drying increases the shelf life and stops microorganisms' growth (Rahimmalek and Goli, 2013). Dried samples also have low water activity, reduced weight and volume for transport and storage, and reduced costs of packaging (Saifullah et al., 2016).

As *S. grandiflora* is rich in bioactive compounds, and nutritional value, and it is important for our daily diet and health. But it is high in moisture contents, perishable and deteriorate in nature. Thus, they cannot keep for long time. Besides it is not available throughout the year. To be preserved for the use in off-season, it needs to be dried and preserved for its phytochemical compounds. Drying is one of the most common methods and cost effective. Therefore, the experiment was conducted with the following objective.

2. Objective

The objective of this study is to investigate the influence of two drying methods (shade drying and oven drying) on the proximate composition and phytochemical content of *S. grandiflora*.

3. Materials and Methods

3.1 Sample Collection

The leaves of S. *grandiflora* were collected from Tatpoe village in Nay Pyi Taw Township, Myanmar. These vegetables were collected in May 2022. The samples were dried and analysed at the Department of Horticulture, and the Department of Postharvest Technology Laboratory, Advanced Center for Agricultural Research and Education, Yezin Agricultural University, Nay Pyi Taw.

3.2 Sample Preparation

Leaves were cleaned to remove dirt on the surface. The leaves were divided into three parts. The first and second parts were used to dry samples for shade and oven. The last part was anlaysed as fresh. For shade drying, the samples were evenly spread on tarpaulin bag for a period of (10-14) days at average ambient temperature (27-30°C). For oven drying, fresh leaves were put into the paper bags and kept in oven at 70°C for 24 hours. Dried leaves were ground into a fine powder. The powder was put in zipper bags and stored in the refrigerator at (2-4°C) for one month before analysis.

3.3 Determination of Proximate Composition

The proximate composition of the samples was determined using Association of Official Analytical Chemist (AOAC, 2005) methods.

3.3.1 Determination of moisture content

Five grams of the sample was weighed into a moisture dish and dried at 105 ± 1°C for 5 hr in the drying oven. After drying, the samples were kept in a desiccator to cool. Drying process was done until a constant weight was obtained. The moisture content was calculated according to following equations.

$$\text{Moisture content (\%)} = \frac{(A - C)}{B} \times 100$$

Where:

B = Sample weight (g)

A= Weight of aluminium dish + sample before drying (g)

C= Weight of aluminium dish + sample after drying (g)

3.3.2 Determination of crude protein content

The crude protein content was determined by using Kjeldhal method. One gram of sample was put into a digestion tube with two digestion tablets. 15 ml of H_2SO_4 were added into the digestion tube. Fifteen samples were digested at 420ºC for 90 minutes. For distillation, 50 ml of 35% NaOH and 50 ml of Boric acid were used and titrated with 0.1 N HCl to obtain the crude protein percentage.

3.3.3 Determination of crude fiber content

The crude fiber content was determined by using fiber analyzer. One gram of sample was weighed and then put into the filter bag. Fifteen bags were soaked into the petroleum ether about 100 ml for 10 minutes. About 2 liters of 1.25% H_2SO_4 solution and 2 liters of 1.25% NaOH solution were used for extraction. And these bags were put in acetone 250 ml and air dry. And then bags were placed in an oven at 105ºC for 2 hours. All filter bags were ignited over the stove until it became gray or white and kept in a muffle furnace at 600ºC for 3 hours.

3.3.4 Determination of crude fat content

The crude fat content was determined by using fat analyzer. Three grams of sample was added into the extraction thimble cup. Petroleum ether (100 ml) was poured into the glass cup. The extraction cup with thimble were placed on the fat analyzer. After finishing the extraction cups were placed at 105℃ for 30 min in an oven and placed in desiccator. The final weight of the extraction cup containing extract was weighed.

3.3.5 Determination of crude ash content

Five gram of sample was weighed in porcelain dish. These dishes were ignited over the stove until the ash turned gray or white obtained. These were kept in a muffle furnace at 600ºC for 3 hours. After that, the ashing dishes were cooled and weighed.

3.3.6 Determination of carbohydrate content

The percentage of total carbohydrate content was determined as follow:

Carbohydrate (%) = 100 – (moisture (%) + crude protein (%) + crude fiber (%) + crude fat (%) + crude ash (%))

3.4 Extraction of Plant Material for Qualitative of Phytochemical

Five grams of leaf powder was put in a sterile bottle and mixed with 25 ml of distilled water. The powders were soaked in it and shaken well and then boiled on water bath at 50-60 ℃ for 30 minutes. The solution was filtered through Whatman No.40 and the filtrate was centrifuged the filtrate at 2500 rpm for 15 minutes. The extract was put in sterile bottles and stored at refrigerator for phytochemical analysis.

3.4.1 Qualitative analysis of phytochemicals

The presence of phytochemicals was determined by using the protocol of Joshi et al., (2013), Shah et al., (2014) and Balamurugan et al., (2019).

Test	Methods	Observation
Alkaloid	1 ml of extract, two drops of Wagner's reagent	reddish brown precipitate
Saponin	5 ml of extract was diluted with 20 ml of water and shaken vigorously	stable froth (foam) up
Tannin	1 ml of extract added a few drops of 5% ferric chloride	blue-green or black
Flavonoid	2 ml of extract added with few drops of 1 N sodium hydroxide solution	intense yellow coloration
Glycoside	0.5 ml of extract mixed about 2 ml of glacial acetic acid and 5% ferric chloride and 1 ml of conc H_2SO_4	brown ring at interface
Terpenoid	3 ml of extract added 1 ml of chloroform and 1.5 ml of conc H_2SO_4 was added along the sides of the tube	reddish brown color
Steroid	2 ml of extract mixed 2 ml of chloroform and 2 ml of conc H_2SO_4	red color and yellowish green fluorescence

3.5 Quantitative analysis of Phytochemicals

3.5.1 Determination of antioxidant activity

The antioxidant activity was determined by DPPH method by Tokala et al., (2021). Stock solution was prepared by dissolving 100 ml of methanol and 24 mg of DPPH (1:4) in an amber volumetric flask and shaken well. Control solution was prepared by mixing 40 ml methanol and 10 ml of DPPH stock solution. About one gram of powder were mixed with 10 ml of methanol and centrifuged at 10,000 rpm at 4°C for 20 min. Then 1 ml of the supernatant was mixed with 2 ml of control solution and incubated in the dark place for 30 minutes. The absorbance was measured at 515 nm by using UV-2600 spectrophotometer. The radical scavenging activity was determined as the radical scavenging percentage based on the following equation:

$$\%\ \text{DPPH scavenging effect (\% of inhibition)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

3.5.2 Determination of vitamin C

The content of vitmin C were determined by using the protocol of Tokala et al., (2021). One gram of sample was mixed with 20 ml of 6 % (w/v) metaphosphoric acid, then homogenized and centrifuged at 5000 rpm at 4˚C for 20 minutes. After that, 200 µl of supernatant was mixed with 200 µl of 3% metaphosphoric acid and 200 µl of Folin-Ciocalteu's (FC) reagent (1:3) ratio and finally added with 1400 µl of distilled water. The mixture samples were kept in dark place for 10 minutes and the absorbance value was measured at 760 nm by using spectrophotometer.

3.5.3 Determination of total phenol content

Folin-Ciocalteu assay was used to determine total phenol compound according to the method of Banerjee, Sanjay, Chethan & Malleshi (2012). (0.5 g) of fine powder was soaked in 10 ml of 80% methanol acidified with 1% HCl. This mixture was heated on water bath at 60°C for 1 hour. Then the extracts were centrifuged at 5000 rpm for 20 minutes. An aliquot (0.1 ml) was mixed with 1.5 ml of 20% (w/v) sodium carbonate. After that, (0.5 ml) of FC reagent was diluted with distilled water in the ratio of 1: 2 (v/v) and incubated for 30 min in dark. The mixture was finally volume made up 10 ml with distilled water. The absorbance was measured at 760 nm by using spectrophotometer.

4. Statistical Analysis

All treatments were replicated three times in Completely Randomized Design. The data were analyzed by using Statistixs software (8^{th} version). Mean comparisons were carried out with LSD (Least Significance Difference) at 5% level.

5. Results and Discussion

All the proximate composition (moisture, crude protein, crude fiber, crude fat, crude ash, and carbohydrate) were highly significantly different ($P<0.01$) among the treatments table 1. Moisture content was the highest (74.90%) in fresh samples followed by shade-dried (12.81%) and oven-dried (11.29%).

The crude protein content ranged from (6.15 % to 22.68%) among the treatments. Shade drying had the highest of crude protein content 22.68%, followed by oven drying 21.12%. The lowest amount of protein percentage was found in fresh samples 6.15%. 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 (Salve, Syed & Shinde, 2020). According to Akah, Eze, & Omah (2017), the shade drying in basil and moringa (Umar et al., 2015) offered high crude protein percentage.

The crude fiber content ranged from (5.17 % to 31.77%) among the treatments. The highest crude fiber content was found in oven drying (31.77%) followed by shade drying (25.69%). The lowest crude fiber percentage was found in fresh sample (5.17%). Umar et al., (2015) also found the highest crude fiber content in moringa and fluted pumpkin by oven drying (60°C) (Obembe, Ojo, & Ileke 2021).

The fresh leaves have crude fat content of (1.37%) whereas (2.69%) was found in shade-dried and (5.44%) in oven-dried. Oven drying demonstrated the highest fat content while the lowest fat content was observed in fresh samples. Similar trends were also observed for crude fat content in oven drying of basil (70°C) Akah, Eze, & Omah, (2017) and moringa (60°C) (Umar et al., 2015).

The crude ash content in fresh samples was lower (2.25%) than those of shade drying (8.97%) and oven drying (8.10%). The shade-dried samples obtained the highest crude ash content among the samples. The lowest crude ash content found in fresh samples. Similar result was found on crude ash content of basil in the shade drying (Akah, Eze, & Omah, 2017).

The carbohydrate content ranged from (10.17 - 27.16%) among the treatments. The shade-dried leaves had the highest carbohydrate content 27.16% and fresh samples had the least (10.17%). The result is in accordance with the study of Obembe, Ojo, & Ileke (2021), where carbohydrate content of fluted pumpkin was the highest in the shade drying.

Table 1. Effect of drying methods on proximate composition of *S. grandiflora*

Treatments	Moisture (%)	Crude protein (%)	Crude fiber (%)	Crude fat (%)	Crude ash (%)	Carbohydrate (%)
Fresh	74.90 a	6.15 c	5.17 c	1.37 c	2.25 c	10.17 c
Shade	12.81 b	22.68 a	25.69 b	2.69 b	8.97 a	27.16 a
Oven	11.29 c	21.12 b	31.77 a	5.44 a	8.10 b	22.29 b
LSD $_{0.05}$	0.60	0.49	1.12	0.29	0.44	1.49
Pr>F	**	**	**	**	**	**
CV (%)	**0.83**	**1.47**	**2.69**	**4.54**	**3.42**	**3.76**

** = significant difference at 1% level

Table 2. Presence of phytochemicals in *S. grandiflora*

Phytochemicals	Fresh	Shade	Oven
Alkaloid	+	+	+
Saponin	+ +	+ +	+
Tannin	+ +	-	-
Flavonoid	±	+	+
Glycoside	-	-	-
Terpenoid	-	+	+
Steroid	-	-	-

(+) indicates presence of phytochemicals, (-) indicates absent of phytochemicals,
(++) indicates high concentration of phytochemicals, (±) indicates doubtful presence of phytochemicals

The presence of the phytochemicals in *S. grandiflora* is presented in table 2. Alkaloid was presnt in all the treatments. Saponin was present in all the treatments, but in high concentration fresh and shade-dried samples. Tannin was absent when dried even though highly concentrated in fresh samples. Schieber et al., (2001) stated that the thermal processing, enzymes, light, and oxygen breakdown the integrity of the cell structure which led to losses of leakage or breakdown on various chemical reactions. The present of flavonoids was found in all treatments but it was doutful in fresh samples. Glycosides and steroids were not found in the treatments. Even though terpenoid was absent in fresh samples, it appeared after drying. The absence or presence of the phytochemical is due to the type of solvent, extraction methods and processing which had variable effect on the content of secondary metabolites (Dirar et al., 2019).

Antioxidant Activity

The antioxidant activity was highly significantly different ($P<0.01$) among the treatments (Table 3). It was maximum in fresh (44.54 %) and shade drying samples (39.09%). Oven drying (12.73%) has minimum antioxidant activity. Roshanak, Rahimmalek & Goli (2016) stated that fresh sample of green tea and sage (Rababah et al., 2015) had high antioxidant content. According to Lim and Murtijaya's (2017), highly loss of antioxidant activity was found in oven drying due to the intense and prolonged thermal treatment effect. Moreover, the decrease in antioxidant activity on drying is related to the degradation of biologically active compounds at high temperatures, due to chemical, enzymatic, or thermal decomposition (Kamiloglu et al., 2016).

Vitamin C

The vitamin C content was highly significantly different ($P<0.01$) among the treatments table 3. Vitamin C content in fresh samples was higher (193.55 mg/100g) than those of shade drying (108.34 mg/100g) and oven drying (12.73 mg/100g). Similarly, the high vitamin C content was obtained in fresh sample of green tea (Roshanak, Rahimmalek & Goli, 2016) and mint, coriander, and curry leaves (Vyankatrao & Commerce, 2014). Vitamin C is heat sensitive and thus

reduced with increasing drying temperature (Igwemma et al., 2013). Vitamin C oxidizes and disappears in high temperatures and long drying times (Jin et al., 2014).

Total Phenol Content (TPC)

The phenol content was highly significantly different (P<0.01) among the treatments (Table 3). The highest TPC was recorded in shade drying (16.41 g/100g) and the lowest was observed in fresh samples (7.47 g/100g). Fresh samples exhibited lower results than that of dried samples. The results are in accordance with the finding in sweet basil (Akah, Eze, & Omah 2017). The increases in the total phenolic content are due to the release of phenolic compounds bound to the plant cell wall (Gumusay et al., 2015).

Table 3. Effect of drying mehthods on antioxidant, vitamin C and total phenol content of *S. grandiflora*

Treatments	Antioxidant activity (%)	Vitamin C (mg/100g)	Total phenol (GAE g /100g)
Fresh	44.54 a	193.55 a	7.47 c
Shade	39.09 b	108.34 b	16.41 a
Oven	12.73 c	115.44 b	10.81 b
LSD $_{0.05}$	**3.63**	**12.98**	**0.80**
Pr>F	**	**	**
CV (%)	**5.66**	**4.67**	**3.46**

** = significant difference at 1% level

6. Conclusion

It was concluded that drying of leaves is the better source of crude protein, crude fat, crude ash, crude fibre, and carbohydrate than the fresh leaves. The highest crude protein, crude ash, and carbohydrate content were observed in shade-drying method. The highest crude fiber and crude fat content were recorded in oven-drying method. Phytochemicals except glycoside and steroid were found in all treatments. Antioxidant activity and vitmin C content were the highest in fresh samples and the lowest in oven-dried samples. The highest amount of total phenol content was found in shade-dried and the lowest was observed in fresh samples. Thus, drying methods are affordable approaches to increase proximate composition and phytochemical content of *S. grandiflora* and to mitigate malnutrition in the developing countries.

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