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Comparative Study of Drying Methods on Proximate Composition, Chlorophyll Retention and Phytochemical properties in *Cnidoscolus aconitifolius* Leaves

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ABSTRACT

Cnidoscolus aconitifolius (Chaya) is a nutrient-rich leafy vegetable widely consumed for its health benefits. However, its high moisture content limits shelf life, making drying the preferred option due to its low cost and suitability for rural settings. This study evaluated the effects of sun drying and the NSPRI parabolic solar dryer on the proximate composition, chlorophyll retention, and phytochemical properties of Chaya leaves to identify drying methods that best preserve their nutritional quality. Fresh leaves were harvested and dried by both methods. Proximate analysis (moisture, ash, fiber, protein, fat, carbohydrate, energy) and chlorophyll content (chlorophyll a, b, and total) were determined using AOAC methods. Phytochemicals including flavonoids, alkaloids, saponins, and tannins were quantified. Data were statistically analyzed by one-way ANOVA at $p<0.05$. Drying significantly reduced moisture from 66.3% in fresh to approximately 7.8% in dried samples, enhancing shelf life. Both drying methods similarly concentrated the existing nutrients by reducing moisture content, and no significant differences were observed in their proximate composition. Chlorophyll retention was significantly higher in solar-dried leaves (49.09±0.21 mg/g total chlorophyll) compared to sun-dried (41.64±0.27 mg/g), indicating better pigment preservation under solar drying. Phytochemical analysis showed significant decreases after drying; however, solar drying preserved higher levels of alkaloids (211.33±1.45 µg/g), saponins (343.67±2.03 µg/g), and tannins (86.0±1.15µg/g) than sun drying, while flavonoids declined similarly (approximately 270 µg/g). These results suggest that solar drying provides a controlled environment that better preserves chlorophyll and phytochemicals, while proximate nutritional quality remains comparable between drying methods. Adoption of solar drying technology is recommended to improve shelf life and maintain quality, particularly in places with limited access to refrigeration.

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1. INTRODUCTION

Cnidoscolus aconitifolius, often known as Chaya or tree spinach, is a leafy vegetable native to tropical climates and is frequently consumed for its nutritional and medicinal qualities (Kuti & Konuru, 2003; Yusuf et al., 2022; Calonico & De La Rosa-Millan, 2023). It is a valuable source of macronutrients, such as carbohydrates, proteins, lipids, and dietary fiber, as well as essential micronutrients, such as vitamins A and C, and minerals which includes potassium, calcium, and iron (Yusuf *et al.*, 2022; Oluka & Nwankwo, 2023). Beyond its nutritional value, *Cnidoscolus aconitifolius* leaves contain a variety of bioactive phytochemicals (phenols, flavonoids, alkaloids, terpenoids, and saponins) that contribute to its antioxidant and therapeutic potential (Yusuf et al., 2022; Oluka & Nwankwo, 2023). These properties have earned the plant the local nickname "Hospital is too far", a phrase that reflects its traditional use in maintaining health and treating ailments (Chukwu et al., 2019; Sarkar et al., 2022).

The preservation of leafy vegetables, such as *Cnidoscolus aconitifolius*, is crucial, especially in countries where fresh produce is seasonal and refrigerated facilities are limited. Drying remains one of the most effective and extensively used postharvest strategies for increasing shelf life by reducing moisture content and preventing microbial development (Fathi et al., 2022). Among drying procedures, sun drying and solar drying are chosen in rural and resource-limited settings due to their low cost and ease of application (Sobowale et al., 2010; Al-Ghadani et al., 2024). However, these drying processes expose the leaves to varying degrees of heat and light, which may result in physicochemical changes, notably changing the proximate composition and the retention of sensitive components such as chlorophyll (Neoh et al., 2016; Singh & Sharma, 2021).

Chlorophyll, responsible for the characteristic green colour of leaves, is not only a significant quality trait but also possesses antioxidant capabilities that contribute to the nutritional value of leafy vegetables (Eze et al., 2020). Loss of chlorophyll during drying can negatively impact both the aesthetic appeal and health benefits of the dried product. Similarly, drying methods can alter the proximate composition, such as moisture, protein, fat, fiber, and carbohydrate content, thereby changing the nutritional quality of the leaves (Yusuf et al., 2022; Ogbuozobe et al., 2024).

Despite the increased interest in *Cnidoscolus aconitifolius* as a functional food, there is limited research on how different drying methods influence its proximate nutrients, phytochemicals, and chlorophyll retention. The retention of phytochemical compounds during drying varies because certain compounds such as vitamin C, some phenolic acids, and specific flavonoids are more heat-labile, while others like tannins and saponins remain relatively stable (Moyo et al., 2023). Some compounds may actually become more concentrated as moisture is removed, while others may undergo degradation or transformation. Understanding these changes is crucial for optimizing drying processes to maximize the retention of beneficial compounds. This study aimed to evaluate the effects of sun and solar drying methods on the proximate composition, phytochemical properties and chlorophyll retention in *Cnidoscolus aconitifolius* leaves. The findings will provide valuable insights for improving postharvest handling and processing techniques to enhance the shelf life and nutritional quality of this important indigenous leafy vegetable.

2. MATERIALS AND METHODS

2.1. Sample Collection and Preparation

Fresh mature leaves of *Cnidoscolus aconitifolius* were harvested from healthy plants cultivated on a local farm in Ido local government area of Oyo State (9° 24'N, 0° 59'W). Leaves were carefully selected to avoid physical damage or disease. The harvested leaves were washed thoroughly under running tap water to remove dirt and impurities, drained for five minutes at ambient temperature and humidity, with excess surface moisture gently blotted using a paper towel to ensure consistency. The cleaned leaves were chopped into approximately 2–3 cm pieces and spread in a single layer to facilitate uniform drying, ensuring adequate airflow around each piece (Salarikia et al., 2016).

2.2. Description of the dryer/drying methods used

Two drying methods were used: sun and solar drying. The solar dryer used for drying was the NSPRI parabolic-shaped solar dryer constructed by the Nigerian Stored Products Research Institute, Nigeria. The dryer had structural dimensions of 6 m × 4 m × 2.8 m, with the longer side facing the East-west direction for maximum reception of solar radiation. It has a black floor that stores heat from the sun and is insulated to prevent heat loss to the ground through conduction. It has a parabolic-shaped structural frame of 2.8 m height, made of galvanized steel pipes. The roof is covered with a transparent UV screen covering material that provided an enclosure and transmitted heat from the sun into the drying chamber. The drying chamber has two drying racks with two layers each made of mild steel angle iron and 28 trays of 0.8 m × 1.0 m made of square pipes and wire mesh. The dryer had an effective area of 22.4 m^2. There are six (6) inlet vents. The top was fitted with two (2) pneumatic aspirators for moisture extraction from the drying chamber. An access door was also provided at one end of the structure (**Figure 1**).

During the drying period, continuous environmental monitoring showed that the solar dryer maintained an average internal temperature of 51.65 °C, compared to an ambient average of 35.50 °C. The internal relative humidity averaged 44.90%, while the ambient relative humidity was 55.49%. The airflow velocity inside the drying chamber averaged 0.57 m/s, compared to an ambient airflow of 1.19 m/s.

Fig. 1: NSPRI Parabolic Solar Dryer used for drying *Cnidoscolus aconitifolius* leaves.

For sun drying, the vegetable leaves were spread in a single layer on clean mesh trays and placed outdoors under direct sunlight between 9:00 AM and 5:00 PM. The leaves were turned intermittently to ensure even drying. Drying was continued until a constant weight was achieved, which required 6–8 hours depending on ambient temperature and relative humidity.

2.3. Determination of Proximate Composition

Proximate composition was determined using AOAC methods: AOAC 934.01 for moisture, AOAC 920.87 for protein, AOAC 923.03 for fat, AOAC 945.38 for ash, AOAC 978.10 for crude fiber (AOAC, 2000). Carbohydrate content was calculated by difference on a dry matter basis.

Thus: Carbohydrate = 100 – (% moisture + % crude fiber + % protein + % lipid + % ash)

2.4. Determination of Caloric Value

The % crude protein, fat, and carbohydrate were used to estimate the calorific value of the sample. Thus, the value of protein content was multiplied by 4; that of lipid by 9 and that of total carbohydrate by 4. The sum of these values gives the calorific value expressed in kcal/100g sample (Dibulo et al., 2017).

2.5. Quantitative determination of Phytochemical Properties

Fresh and dried leaves were ground into a powder using a mechanical grinder, and a known weight 10g of the ground leaves was macerated in 1 L of absolute ethanol using a maceration flask. The mixture was left for 72 hours with periodic stirring and then filtered using a muslin cloth to remove fine residues. The filtrate was then concentrated using a rotary evaporator at 45°C to obtain a crude ethanol extract, which was stored in a labeled, sterile screw-capped bottle.

2.6. Determination of Alkaloids

Alkaloids were determined following Dibulo et al. (2017) with a temperature adjustment to prevent degradation at ≤60 °C. A 5 g sample was dissolved in 100 mL of 20% acetic acid in ethanol, covered, and left to stand for 4 hours at 25 °C. The mixture was filtered, and the filtrate was concentrated in a water bath to one-quarter of its original volume. Concentrated ammonium hydroxide was added drop wise until precipitation was complete. The precipitate was washed with 1% NH_4OH, filtered, dried to constant weight, cooled, and reweighed. Alkaloid content was expressed as a percentage of sample weight.

2.7. Determination of Flavonoids

A 10 g sample was repeatedly extracted with 100 mL of 80% methanol at room temperature. The filtrate was evaporated to dryness in a water bath, and the residue was weighed. flavonoid content was expressed on a dry-weight basis using moisture values from proximate analysis (Dibulo et al., 2017).

2.8. Determination of Saponins

The sample (5 g) was dissolved in 100ml of 20% acetic acid in ethanol and allowed to stand in a water-bath at 50°C for 24 hours. The mixture was filtered and the extract was concentrated using the water-bath to one-quarter of the original volume. Concentrated NH4OH solution was added drop-wise to the extract until the precipitation was complete. The solution was allowed to settle and the precipitate was collected by filtration, oven-dried and weighed (Dibulo et al., 2017). Saponin content was expressed as a percentage of sample weight.

2.9. Determination of Total Tannin

Tannin content was determined using the Folin–Denis method (Pearson, 1974). A 10 g sample was defatted with 100 mL petroleum ether for 24 hours. The residue was air-dried to remove solvent and re-extracted with 100 mL of 10% acetic acid in ethanol for 4 hours. After filtration, 25 mL of NH_4OH was added to precipitate tannins, and the mixture was gently heated to remove excess ammonia.

A 2 mL aliquot of the extract was diluted with 20 mL ethanol and titrated with 0.1 M NaOH using phenolphthalein as indicator. Tannin content was calculated and expressed as % tannic acid equivalent.

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2.10. Estimation of Chlorophyll

Healthy fresh green and dried leaves of *Cnidoscolus aconitifolius* were collected, and 1 g of each was weighed. For the extraction of chlorophyll, 1 g of the sample each were ground to a fine pulp with the addition of 20 ml of 80% acetone with a mortar and pestle. This paste was then centrifuged for 5 min at 5000 rpm. The supernatant was transferred to a 100 ml beaker. The residue was then ground with 20 ml of 80% acetone, centrifuged for 5 min at 5000 rpm, and the supernatant was transferred to the same beaker. The residue was repeatedly extracted until nearly colourless, and all supernatants were combined. The extract volume was made up to 100 mL with 80% acetone. The absorbance of the extract solutions was read at 645 and 663 nm against the solvent (80% acetone) blank. The average value of triplicate determination was expressed as mg of chlorophyll per gram of the leaf tissue. The amount of chlorophyll present in the extract, i.e., mg of chlorophyll present per gram of tissue, was calculated using the following equations (Priyadharshana et al., 2022):

$$\text{mg of chlorophyll} - \text{A per gram of tissue} = [12.7(A_{663}) - 2.69(A_{645})] \times \text{V} \div (1000 \times \text{W})$$

$$\text{mg of chlorophyll} - \text{B per gram of tissue} = [22.9(A_{645}) - 4.68(A_{663})] \times \text{V} \div (1000 \times \text{W})$$

$$\text{mg of total chlorophyl per gram of tissue} = [20.2(A_{645}) + 8.02(A_{663})] \times \text{V} \div (1000 \times \text{W})$$

Where A = absorbance at specific wavelengths, V = final volume of chlorophyll extract in 80% acetone, which in this case is 100 ml, and W = fresh weight of tissue extracted, which is 1 g.

2.11. Statistical Analysis

All proximate, chlorophyll, and phytochemical analyses were performed in triplicate, and results are presented as mean ± standard error of the mean (SEM). Statistical comparisons were conducted using one-way analysis of variance (ANOVA) with treatment as a single factor comprising three levels: fresh leaves, sun-dried, and solar-dried samples. Post hoc comparisons were performed using Tukey's honestly significant difference (HSD) test at a significance level of $p < 0.05$. All analyses were performed using SPSS Statistics version 25.

3. RESULTS AND DISCUSSION

3.1. Effect of Drying Methods on Proximate Composition of Fresh and Dried *Cnidoscolus aconitifolius* leaves

Table 1 presents the results for proximate composition of fresh as dried *Cnidoscolus aconitifolius* leaves as affected by drying method. The moisture content decreased drastically from 66.33% in fresh leaves to 7.82% and 7.33% in sun- and solar-dried leaves, respectively. This significant reduction ($p < 0.05$) demonstrates the effectiveness of both drying methods in removing water, a key factor for extending shelf life and ensuring microbial stability. Similar moisture reductions have been reported for *Cnidoscolus aconitifolius* and other leafy vegetables subjected to drying (Chukwu et al., 2019; Eze et al., 2020).

Ash content, an indicator of total mineral content, increased significantly upon drying, reaching 9.33% in solar-dried leaves and 7.67% in sun-dried leaves, compared to 0.93% in fresh leaves (wet basis). This increase reflects a dry matter concentration effect due to moisture loss rather than the formation of new minerals (Busari et al., 2022). Comparable results have been reported in dried *Cnidoscolus aconitifolius* (Yusuf et al., 2022). Crude fiber content also rose significantly after drying, particularly in sun-dried leaves (11.33%) compared to solar-dried (11%) and fresh leaves (6.37%). However, the difference between the two drying methods was not statistically significant ($p > 0.05$). Similar increases have been observed in leafy greens, enhancing their dietary fiber value (Emelike et al., 2020).

Protein content increased from 9.19% in fresh leaves to 11.20% in sun-dried and 12.67% in solar-dried leaves, with both dried samples significantly higher than fresh leaves ($p < 0.05$). This apparent increase is due to concentration following moisture removal (Eze et al., 2020), and there was no significant difference between the two drying methods ($p > 0.05$). Fat content remained relatively unchanged, ranging from 5.0–6.3% across fresh and dried samples, showing no significant changes ($p > 0.05$). This aligns with previous reports indicating that lipids in leafy vegetables are relatively stable under drying conditions (Garba & Oviosa, 2019). Carbohydrate content and energy value increased markedly in dried samples. Carbohydrates rose from 10.87% in fresh leaves to 56.98% and 54.67% in sun-dried and solar-dried respectively, while energy content increased from 137 kcal in fresh to 317.72 kcal and 314.3 kcal in sun-dried and solar-dried respectively. It is important to note that the increase in nutrient percentages is due to moisture reduction rather than actual synthesis or addition of nutrients, which is typical in postharvest dehydration processes as observed in related studies on dried leafy vegetables (Eze et al., 2020; Busari et al., 2022). There was no significant change between the sun dried and solar dried sample. This shows that the drying effect did not significantly affect the proximate composition of the leafy vegetable dried.

Table 1: Proximate composition of fresh and dried leaves of Cnidoscolus aconitifolius

Parameter	Drying Methods		
	Fresh	Sun drying	Solar drying
Moisture (%)	66.33^{a}±1.33	7.82^{b}±0.16	7.33^{b}±0.67
Ash (%)	0.93^{b}±0.07	7.67^{a}±0.33	9.33^{a} ±2.33
Fiber (%)	6.37^{b}±0.38	11.33^{a}±0.33	11.00^{a}±1.00
Protein (%)	9.19^{b}±0.54	11.20^{a}±0.35	12.67^{a}±0.42
Fat (%)	6.30^{a}±0.12	5.00^{a}±1.00	5.00^{a}±0.58
CHO (%)	10.87^{b}±1.97	56.98^{a}±0.75	54.67^{a}±0.92
Energy (kcal)	136.97^{b}±6.83	317.72^{a}±5.34	314.33^{a} ±7.44

Values are presented as mean±SEM. Means with different superscripts in each column are significantly different (P<0.05).

3.2. Effect of Drying Methods on Chlorophyll Retention of fresh and dried *Cnidoscolus aconitifolius leaves*

The fresh leaves had the highest mean chlorophyll values on a wet weight basis: 27.93 mg/g for chlorophyll a, 29.95 mg/g for chlorophyll b and 57.94 mg/g for total chlorophyll. The solar-dried samples retained more chlorophyll content for chlorophyll a, chlorophyll b and total chlorophyll (23.12, 25.86 and 49.09 mg/g, respectively) than sun-dried samples with 21.84, 19.74 and 41.64 mg/g for chlorophyll a, chlorophyll b and total chlorophyll, respectively (**Table 2**). There was a significant difference (p<0.05) among the samples, confirming the effect of drying conditions on pigment retention.

The findings of this study are consistent with previous research on *Cnidoscolus aconitifolius* and other green leafy vegetables. Eze et al. (2020) reported a significant decline in chlorophyll content of *Cnidoscolus aconitifolius* leaves after sun and oven drying, with sun drying causing the greatest loss. Also, Busari et al. (2022) found that sun drying of wild lettuce (*Lactuca taraxacifolia*) resulted in a marked reduction in chlorophyll compared to solar and shade drying, attributing the loss to the combined effects of heat and direct solar radiation. The values observed in this study for fresh leaves are within the range reported for other dark green leafy vegetables, such as *Amaranthus hybridus* and *Telfairia occidentalis*, which typically contain 20 to 40 mg/g total chlorophyll on a dry weight basis (Patel et al., 2022). The higher values in fresh *Iyana paja* may reflect varietal differences, growing conditions, or leaf maturity at harvest.

The mechanism behind chlorophyll degradation during drying is well established: chlorophylls are highly sensitive to heat, light, and oxygen, leading to conversion to pheophytins and other degradation products (Singh & Sharma, 2021). The controlled environment of the solar dryer (PSSD) likely reduced exposure to extreme temperatures and UV radiation, resulting in higher chlorophyll retention compared to open sun drying.

3.3 Effect of drying methods on phytochemical properties of *Cnidoscolus aconitifolius leaves*

The effect of drying methods on the phytochemical properties of *Cnidoscolus aconitifolius* leaves is presented in **Table 3**, showing significant reductions ($p < 0.05$) in flavonoids, alkaloids, saponins, and tannins after drying. Fresh leaves were measured on a wet weight basis, while dried samples are expressed on a dry weight basis. Values are reported as mean ± SEM of three replicates. Specifically, flavonoid content declined from 458.00 µg/g in fresh leaves to 271.10 µg/g and 270.67 µg/g in sun-dried and solar-dried leaves, respectively with no significant difference between the dried samples (p> 0.05). This reduction is caused by heat- and light-induced degradation of flavonoids during drying, as these compounds are sensitive to oxidative damage and thermal stress (Xu et al., 2024).

However, the alkaloid levels dropped from 577.33 µg/g in fresh leaves to 182.67 µg/g and 211.33 µg/g in sun-dried and solar-dried leaves, respectively, with solar drying preserving significantly more alkaloids than sun drying. This result is in agreement with studies indicating that less intense and controlled drying environments, such as solar drying, reduce enzymatic oxidation and photo-degradation of alkaloids, thereby enhancing retention of these bioactive metabolites (Natumanya et al., 2021). Also, saponin content decreased as well, from 351.33 µg/g in fresh leaves to 327.44 µg/g and 343.67 µg/g for sun and solar drying, respectively. Previous research attributes this partial retention to the relatively higher thermal stability of saponins compared to other phytochemicals, which is why solar drying, with its milder conditions, preserves these compounds better (Sharma et al., 2023). This is consistent with observed trends in medicinal plants where saponins demonstrate resistance to moderate heat treatment.

There was a decline in the tannins level caused as a result of the drying, a high reduction from 380.67 µg/g in fresh samples to 49.33 µg/g and 86.00 µg/g in sun and solar drying respectively. The pronounced difference between sun and solar drying for tannins and alkaloids can be attributed to the higher susceptibility of their chemical structures to thermal and light-induced degradation, whereas flavonoids, being more stable polyphenols, showed no significant difference. The higher retention in solar drying reflects the controlled temperature and reduced UV exposure compared to open sun drying (Chisowa, 2022). The higher tannin retention observed with solar drying compared to sun drying supports the view that moderated drying parameters better maintain sensitive antioxidant compounds

Table 2: Effect of drying methods on chlorophyll retention of *Cnidoscolus aconitifolius leaves*

Drying Method	Chlorophyll A	Chlorophyll B	Total Chlorophyll
Fresh	27.93^{a}±0.17	29.95^{a}±0.16	57.94^{a}±0.27
Sun drying	21.84^{c}±0.10	19.74^{c}±0.18	41.64^{c}±0.27
Solar drying	23.12^{b}±0.14	25.86^{b}±0.18	49.09^{b}±0.21

Values are presented as mean±SEM. Means with different superscripts in each column are significantly different (P<0.05).

Table 3: Effect of drying methods on phytochemical properties of *Cnidoscolus aconitifolius leaves*

Method	Flavonoid (µg/g)	Alkaloid(µg/g)	Saponins (µg/g)	Tannins (µg/g)
Fresh	458.00^{a}±1.53	577.33^{a}±1.76	**351.33^{a}±1.20**	**380.67^{a}±1.20**
Sun drying	271.10^{b}±1.45	182.67^{c}±1.45	327.44^{c}±2.03	**49.33^{c}±0.88**
Solar drying	270.67^{b}±1.45	211.33^{b}±1.45	**343.67^{b}±2.03**	**86.00^{b}**±1.15

Values are presented as mean±SEM. Means with different superscripts in each column are significantly different (P<0.05).

CONCLUSION

This comparative study demonstrates that both sun and solar drying effectively reduce moisture content in *Cnidoscolus aconitifolius* leaves, thereby enhancing shelf life while significantly concentrating proximate nutrients such as protein, ash, fiber, carbohydrates, and energy value due to moisture loss. However, solar drying shows better retention of chlorophyll and key phytochemicals including alkaloids, saponins, and tannins compared to sun drying, likely attributable to its controlled drying environment that minimizes heat and light-induced degradation. Although flavonoids are similarly reduced by both methods, the overall preservation of bioactive compounds and vibrant green colour was better maintained by solar drying, suggesting it as a preferable method for processing *Cnidoscolus aconitifolius* leaves to retain their antioxidant properties and consumer acceptability.

As a result, solar drying is the better drying technique for Cnidoscolus aconitifolius since it balances efficient dehydration with the preservation of important nutrients and phytochemicals. Future studies could assess the effects on retained phytochemicals' bioavailability and functional effectiveness and sensory attributes.

CONFLICT OF INTEREST

All authors declare that they do not have any conflicts of interest that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY

The data used to support the findings of this study are available upon reasonable request from the corresponding author.

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