



Comparative Phytochemical and Proximate Composition of Mature and Sprout Shoots of *Anacardium occidentale* Leaves

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Abstract

This study comparatively evaluated the proximate composition and phytochemical profiles of sprout and mature leaves of *Anacardium occidentale* L. Fresh leaf samples were collected, authenticated, air-dried, pulverized, and separately extracted with ethanol by maceration. Proximate composition was determined using standard AOAC procedures, while qualitative phytochemical screening and quantitative phytochemical analyses were performed using established methods. Mature leaf extract (MLE) exhibited higher protein (16.90%), ash (7.83%), crude fibre (18.40%), and carbohydrate (48.90%) contents than sprout leaf extract (SLE), whereas SLE had substantially higher moisture content (18.12%). Fat content did not differ significantly between the samples. Qualitative screening showed that both extracts contained alkaloids, flavonoids, tannins, saponins, phenols, glycosides, terpenoids, and reducing sugars. Steroids, anthraquinones, and cardiac glycosides were detected only in SLE. Quantitatively, MLE showed higher phenolics, flavonoids, tannins, saponins, alkaloids, glycosides, terpenoids, and reducing sugars, while steroids, anthraquinones, and cardiac glycosides occurred exclusively in SLE. Overall, maturation significantly influenced the chemical and nutritional characteristics of *A. occidentale* leaves.

Keywords: *Anacardium occidentale*; Sprout leaves; Mature leaves; Phytochemical composition; Proximate composition.

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1. Introduction

Anacardium occidentale L. (cashew), belonging to the family Anacardiaceae, is an economically important tropical plant widely cultivated for its edible nuts and other valuable products (Mitchell *et al.*, 2022; Asna and Menon, 2024). Beyond the nut, different parts of the plant, including

leaves and shoots, have attracted scientific interest because they contain nutritionally important constituents and diverse secondary metabolites (Palei *et al.*, 2020). Cashew leaves have been reported to contain phenolic compounds, flavonoids, tannins, terpenoids, saponins, and other phytochemicals associated with various biological activities, including antioxidant and antimicrobial effects. These properties indicate that cashew leaves may represent a valuable but underutilized source of bioactive compounds (Malik and Bhadauria, 2020; Aourabi *et al.*, 2021; Ouahabi *et al.*, 2024; John *et al.*, 2026)

Phytochemical composition can vary considerably by plant species, geographical location, environmental conditions, processing methods, and particularly the developmental stage of the plant tissue (Isah, 2019; Li *et al.*, 2020; Pant *et al.*, 2021; Chetouani *et al.*, 2023; Kabir *et al.*, 2025). Environmental and physiological factors can influence the biosynthesis, accumulation, and distribution of plant secondary metabolites, resulting in considerable variation in phytochemical profiles among plant tissues and growing conditions (Isah, 2019; Li *et al.*, 2020; Kabir and Lawan, 2025). Processing and drying conditions may also affect the concentration and relative abundance of phytochemicals in *Anacardium occidentale* leaves (Bayuwana *et al.*, 2020). The transition from sprout shoots to mature leaves involves substantial physiological and metabolic changes that may influence the synthesis and accumulation of secondary metabolites (Tajidin *et al.*, 2019; Guo *et al.*, 2020).

Comparative metabolomic studies have demonstrated clear differences in metabolite composition between young and mature leaves, including changes in phenolics, flavonoids, terpenoids, and other specialized metabolites (Rao *et al.*, 2021; Zhang *et al.*, 2022). Consequently, mature and sprout shoots of *A. occidentale* may differ in their qualitative and quantitative phytochemical profiles. Comparative assessment of these tissues is therefore important for determining whether developmental stage influences the abundance of potentially valuable phytochemicals (Costa *et al.*, 2020; Tajidin *et al.*, 2019).

Qualitative phytochemical screening provides preliminary information on the classes of secondary metabolites present in plant materials, whereas quantitative analysis provides information on their relative abundance and concentration (Silva *et al.*, 2020; Nagori and Rajput, 2025; Kabir and Lawan, 2026). Phytochemicals such as phenolics, flavonoids, tannins, alkaloids, saponins, terpenoids, steroids, and glycosides are of considerable scientific interest because of their diverse biological and pharmacological properties (Santini *et al.*, 2019; Silva *et al.*, 2020; Shen *et al.*, 2022; Santini *et al.*, 2019). Phenolic compounds constitute an important group of plant secondary metabolites with antioxidant, antimicrobial, anti-inflammatory, and other biological activities, making them relevant to both pharmaceutical research and food applications (Cosme *et al.*, 2020; Martins *et al.*, 2020). Flavonoids similarly represent a major class of plant bioactive compounds whose biological activities and potential therapeutic applications have received considerable scientific attention (Shen *et al.*,

2022). Quantitative characterization of these compounds can therefore provide a stronger basis for comparing mature and sprout tissues and identifying the developmental stage with greater potential for pharmaceutical, nutraceutical, or functional-food applications (Silva *et al.*, 2020; Maqsood *et al.*, 2020; Giampieri & Battino, 2020).

In addition to phytochemicals, proximate composition provides essential information about the nutritional characteristics of plant materials. Parameters such as moisture, ash, crude protein, crude fat, crude fibre, and carbohydrate reflect major nutritional components and can help determine the potential value of plant tissues for food, feed, or other applications (Perović *et al.*, 2021; Gupta *et al.*, 2019). The nutritional and phytochemical composition of edible plant materials can vary according to plant variety, environmental conditions, cultivation practices, and the particular plant tissue examined (Gupta *et al.*, 2019; Qaderi *et al.*, 2023). Studies on *Anacardium occidentale* leaves have demonstrated the occurrence of several bioactive phytochemical groups together with measurable nutritional components, supporting the potential value of cashew leaves as a source of bioactive and nutritional constituents (Bayuwana *et al.*, 2020; Oloruntola, 2021). Furthermore, recent investigations of cashew biomass have identified phenolics and other bioactive compounds in leaf extracts and demonstrated their potential biological activities (Costa *et al.*, 2020; Savi *et al.*, 2024). Although *A. occidentale* has been investigated extensively for its nutritional and medicinal properties, comparatively less attention has been given to the relationship between developmental stage and the combined phytochemical and proximate characteristics of its mature and sprout shoots.

Therefore, comparative investigation of mature and sprout shoots of *A. occidentale* leaves is important for establishing how developmental stage influences their chemical and nutritional characteristics. Plant developmental stage can substantially affect the accumulation and distribution of specialized metabolites, with young and mature leaves frequently exhibiting distinct metabolic profiles (Guo *et al.*, 2020; Tajidin *et al.*, 2019). Differences in metabolite composition between young and mature tissues have also been demonstrated in other plant species, indicating that developmental stage can influence the relative abundance of primary and secondary metabolites (Rao *et al.*, 2021). In addition, harvesting time can determine the concentration of specific secondary metabolites because different compounds may reach maximum accumulation at different developmental stages (Pérez *et al.*, 2024). Such developmental variation is important when selecting plant tissues for pharmaceutical, nutraceutical, and food-related applications because the concentration of bioactive constituents may influence their functional properties (Maqsood *et al.*, 2020; Giampieri and Battino, 2020). This study aimed to comparatively evaluate the qualitative and quantitative phytochemical profiles and proximate composition of mature and sprout shoots of *Anacardium occidentale* leaves. The findings may provide useful information for identifying the more phytochemically and nutritionally valuable developmental

stage and contribute to the potential utilization of cashew leaf materials as sources of bioactive compounds and functional nutritional resources.

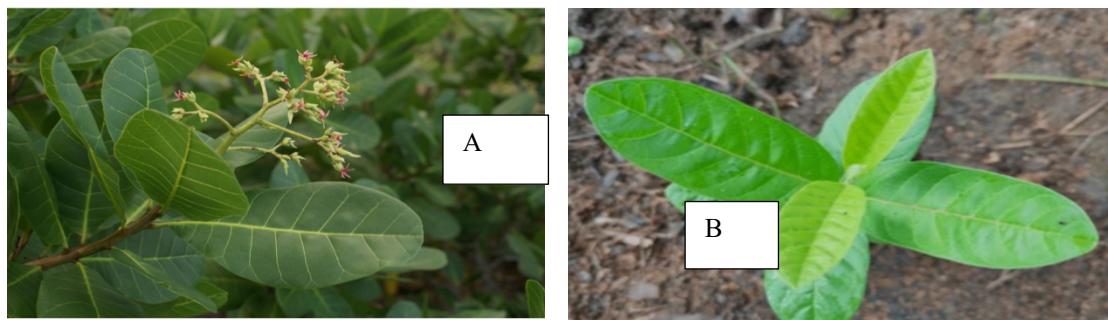


Figure 1: Mature (A) and sprout (B) shoots of *anacardium occidentale*

2. Methodology

2.1 Collection, Authentication and Preparation of Plant Materials

Fresh sprout and mature leaves of *Anacardium occidentale* L. were collected from healthy, disease-free trees within the premises of the Federal Polytechnic Idah, Kogi State, Nigeria, during the early morning to minimize moisture loss and potential degradation of bioactive constituents. The plant material was authenticated by Botanist at the National Institute for Pharmaceutical Research and Development (NIPRD) Herbarium, Idu, Abuja, Nigeria, and assigned voucher specimen number NIPRD/H/7561. A voucher specimen was deposited at the herbarium for future reference (Sharma *et al.*, 2025).

The collected leaves were thoroughly washed with running tap water to remove adhering soil and debris and subsequently air-dried at ambient temperature (25–30 °C) under laboratory conditions away from direct sunlight. The dried sprout and mature leaves were separately pulverized into fine powders using a mechanical grinder and stored in labelled airtight polyethylene containers at room temperature until extraction and analysis (Sharma *et al.*, 2025).

2.2 Extraction Procedure

Six hundred grams (600 g) each of powdered sprout and mature *A. occidentale* leaves were separately macerated in ethanol in labelled reagent bottles. The extraction was conducted at room temperature for six days with regular agitation. The resulting mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated to dryness using a water bath. The resulting dark-green extracts were transferred into labelled containers and stored under refrigerated conditions until further analyses. The extracts from sprout and mature leaves were subsequently designated sprout leaf extract (SLE) and mature leaf extract (MLE), respectively (Dagdag *et al.*, 2024; Abubakar and Haque, 2019).

2.3 Proximate Composition

The proximate composition of the sprout and mature leaf samples was determined using standard Association of Official Analytical Chemists (AOAC) procedures. Moisture, ash, crude protein, crude fibre, crude fat, and carbohydrate contents were determined (Kamboj and Nanda, 2018).

Moisture content was determined by oven-drying weighed samples at 105 °C to constant weight. Ash content was determined by dry ashing in a muffle furnace at approximately 550 °C until a constant ash weight was obtained. Crude protein was determined by the Kjeldahl method through acid digestion, alkalization, steam distillation, and titration of liberated ammonia. Total nitrogen was converted to crude protein using a conversion factor of 6.25 (Liu *et al.*, 2021; Ahmed *et al.*, 2022).

Crude fat was determined by Soxhlet extraction using petroleum ether (40–60 °C) as the extraction solvent. Crude fibre was determined following sequential digestion of the defatted sample with dilute sulfuric acid and sodium hydroxide, followed by drying and ashing of the residue. Carbohydrate content was estimated by difference using the nitrogen-free extract equation:

$$[\%NFE = 100 - (\%Moisture + \%Ash + \%Protein + \%Fat + \%Fibre)] \dots\dots\dots 1$$

All analyses were performed using replicate determinations, and results were expressed on an appropriate dry-weight basis.

2.4 Qualitative Phytochemical Screening

Qualitative phytochemical screening of SLE and MLE was performed using established phytochemical procedures to determine the presence of major secondary-metabolite classes. The extracts were screened for alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, polyphenols, and glycosides (Bayuwana *et al.*, 2020).

Alkaloids were screened using Mayer's reagent, with characteristic precipitate formation considered positive. Terpenoids and steroids were assessed using Salkowski-type reactions based on characteristic colour development at the solvent–acid interface. Tannins and polyphenols were evaluated using ferric chloride and related colour/precipitation reactions. Saponins were detected using the froth test, while flavonoids were assessed using an appropriate colour-reaction test. Glycosides were evaluated using a standard qualitative glycoside assay. Results were recorded according to the presence or absence of characteristic reactions (Costa *et al.*, 2020).

2.5 Quantitative Phytochemical Analysis

Quantitative phytochemical analysis was performed spectrophotometrically using standard analytical procedures. Two grams (2 g) of each powdered sample were extracted with distilled water,

filtered, and made up to a known volume in volumetric flasks. Appropriate aliquots of the filtrates were transferred into cuvettes, and the respective standards and reagent blanks were prepared. The spectrophotometer was calibrated using the appropriate blank and standard solutions before measurement (Gonfa *et al.*, 2020)

The concentrations of the investigated phytochemical constituents were calculated from their respective calibration curves and expressed in appropriate equivalent units.

3. Results and Discussion

3.1 Proximate Composition of the SLE and MLE

Table 1 shows the proximate composition of sprout leaves (SLE) and mature leaves (MLE) of *Anacardium occidentale*. SLE recorded a significantly higher moisture content ($18.12 \pm 0.20\%$) than MLE ($4.87 \pm 0.10\%$), indicating greater water retention in the younger leaves. In contrast, MLE had significantly higher protein ($16.90 \pm 0.13\%$), ash ($7.83 \pm 0.10\%$), crude fibre ($18.40 \pm 0.14\%$), and carbohydrate ($48.90 \pm 0.10\%$) contents than SLE, which recorded $12.40 \pm 0.10\%$, $5.23 \pm 0.08\%$, $15.57 \pm 0.17\%$, and $46.48 \pm 0.02\%$, respectively. These differences suggest that maturation is associated with increased accumulation of proteins, minerals, structural fibre, and carbohydrates in the leaves.

Table 1: Comparative proximate composition of the SLE and MLE

Component	SLE (%)	MLE (%)
Moisture	18.12 ± 0.20^a	4.87 ± 0.10^b
Protein (Kjeldahl $\times 6.25$)	12.40 ± 0.10^b	16.90 ± 0.13^a
Fat (Soxhlet)	2.20 ± 0.07^a	3.10 ± 0.05^a
Ash (550 °C)	5.23 ± 0.08^b	7.83 ± 0.10^a
Crude fiber	15.57 ± 0.17^b	18.40 ± 0.14^a
Carbohydrate	46.48 ± 0.02^b	48.90 ± 0.10^a

The fat content was slightly higher in MLE ($3.10 \pm 0.05\%$) than in SLE ($2.20 \pm 0.07\%$); however, the identical superscript letter indicates that this difference was not statistically significant. Overall, the findings demonstrate that the developmental stage of *A. occidentale* leaves influences their proximate composition, with mature leaves generally showing a higher concentration of major

nutritional components, while sprout leaves contain considerably more moisture. The higher fibre and ash contents of mature leaves may reflect increased cell-wall development and mineral accumulation during leaf maturation.

3.2 Qualitative Phytochemical Screening of the SLE and MLE

Table 2: Comparative qualitative phytochemicals screening of the SLE and MLE

S/n	Phytochemicals	SLE	MLE
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Tannins	+	+
4	Saponins	+	+
5	Phenols	+	+
6	Glycosides	+	+
7	Terpenoids	+	+
8	Steroids	+	-
9	Anthraquinones	+	-
10	Cardiac Glycosides	+	-
11	Phlobatannins	-	-
12	Resins	-	-
13	Reducing Sugars	+	+

The qualitative phytochemical screening revealed that both sprout leaf (SLE) and mature leaf (MLE) samples of *Anacardium occidentale* contained several important secondary metabolites. Alkaloids, flavonoids, tannins, saponins, phenols, glycosides, terpenoids, and reducing sugars were detected in both SLE and MLE. These phytochemicals are associated with various biological activities, including antioxidant, antimicrobial, anti-inflammatory, and protective effects, suggesting that both developmental stages may possess considerable pharmacological potential.

However, some differences were observed between the two samples. Steroids, anthraquinones, and cardiac glycosides were detected in SLE but were absent in MLE, indicating that the production or accumulation of these metabolites may be influenced by leaf developmental stage. Phlobatannins and resins were not detected in either sample. Overall, the presence of a broad range of phytochemicals in both sprout and mature leaves demonstrates that *A. occidentale* leaves are chemically diverse, while the additional metabolites detected in the sprout leaves suggest that younger tissues may contain certain secondary metabolites that decline or become undetectable as the leaves mature.

3.3 Quantitative Phytochemical Screening of the SLE and MLE

Table 3: Quantitative phytochemical analysis of the SLE and MLE

S.N	Phytochemical	SLE	MLE
1	Total phenolic acid (as Gallic acid equivalents) mg GAE/g extract)	42.50	78.30
2	Total flavonoids (as Quercetin equivalents) (mg QE/g extract)	22.70	36.10
3	Tannins mg (TAE/g extract)	12.20	24.80
4	Saponins (% w/w)	3.20	6.80
5	Alkaloids (% w/w)	0.95	2.10
6	Glycosides (% w/w)	2.40	3.10
7	Terpenoids (% w/w)	3.80	4.00
8	Steroids (% w/w)	2.40	-
9	Anthraquinones (mg/g)	1.41	-
10	Cardiac glycosides (mg/g)	0.5	-
11	Phlobatannins (% w/w)	-	-
12	Resins	-	-
13	Reducing sugar	1.58	2.35

The quantitative phytochemical analysis revealed that mature leaf extract (MLE) generally contained higher concentrations of the major phytochemicals than sprout leaf extract (SLE). Total phenolic content increased from 42.50 mg GAE/g extract in SLE to 78.30 mg GAE/g extract in MLE, while total flavonoids increased from 22.70 to 36.10 mg QE/g extract. Similarly, tannins increased from 12.20 to 24.80 mg TAE/g, saponins from 3.20 to 6.80%, alkaloids from 0.95 to 2.10%, glycosides from 2.40 to 3.10%, and terpenoids showed a slight increase from 3.80 to 4.00%. Reducing sugars also increased from 1.58 to 2.35% in MLE. These findings suggest that maturation may promote the accumulation of several important secondary metabolites in *Anacardium occidentale* leaves.

In contrast, steroids, anthraquinones, and cardiac glycosides were detected only in SLE, with concentrations of 2.40%, 1.41 mg/g, and 0.50 mg/g, respectively, while they were not detected in MLE. Phlobatannins and resins were absent from both developmental stages. The higher levels of phenolics, flavonoids, tannins, saponins, and alkaloids in MLE may contribute to greater antioxidant and other biological activities, whereas the presence of steroids, anthraquinones, and cardiac glycosides exclusively in SLE demonstrates that leaf developmental stage can substantially influence the phytochemical profile. Overall, the results indicate both quantitative and qualitative changes in phytochemical composition during maturation of *A. occidentale* leaves.

Phytochemical composition provides important insight into the biological potential and ecological functions of plant materials. The occurrence of phenolics, flavonoids, tannins, saponins, alkaloids, terpenoids and other secondary metabolites reflects the chemical defence capacity of plants against herbivores, pathogens and environmental stressors. Phenolic compounds, particularly flavonoids, are of considerable interest because their structural characteristics enable radical scavenging and metal-chelating activities, which can contribute to the antioxidant properties of plant extracts (Shen *et al.*, 2022; Plaskova & Mlcek, 2023; Amayindi *et al.*, 2024; Danjuma *et al.*, 2025). Recent evidence also indicates that phenolics, terpenes and alkaloids possess diverse biological activities, including antioxidant and anti-inflammatory effects, although their activities are strongly dependent on molecular structure, concentration and interactions among constituents. Therefore, differences in phytochemical concentrations between young and mature plant tissues may reflect ontogenetic changes in secondary metabolism, resource allocation and plant defence strategies. Such variation may also be influenced by genotype, environmental conditions, geographical origin, harvesting period and extraction methodology. In particular, extraction conditions can substantially affect the recovery of phenolic and other bioactive compounds because these metabolites differ in polarity, stability and association with the plant matrix (Kumar *et al.*, 2021; 2021; Kabir and Lawan, 2024). Consequently, quantitative differences observed among plant tissues should be interpreted in relation to both biological development and methodological factors rather than solely as evidence of superior or inferior phytochemical quality.

Proximate composition complements phytochemical characterization by providing information on the major nutritional constituents of plant materials, including moisture, ash, crude protein, crude fat, crude fibre and carbohydrate. Moisture content is particularly important because it influences the stability and storage quality of plant materials, whereas ash provides an overall estimate of the inorganic or mineral fraction. Protein contributes to the nutritional value of the material, while lipids represent an energy-dense fraction and may facilitate the absorption of fat-soluble constituents. Crude fibre is associated with structural components of plant tissues and contributes to dietary bulk, whereas carbohydrates constitute an important source of metabolic energy. Proximate composition provides important information on the nutritional and physicochemical characteristics of *Anacardium occidentale* leaves. Previous studies have demonstrated that cashew leaves contain appreciable levels of crude protein, crude fibre, crude fat, ash and carbohydrate, although the reported values vary considerably depending on plant age, geographical location, environmental conditions and sample preparation (Oloruntola, 2021; Folasade *et al.*, 2022). In particular, *A. occidentale* leaf powder has been reported to contain 14.65% crude protein, 16.95% crude fibre, 10.81% crude fat, 3.70% ash and 49.58% nitrogen-free extract, highlighting its potential nutritional value (Oloruntola, 2021). Similarly,

phytochemical investigations have demonstrated the presence of flavonoids, tannins, alkaloids, saponins and terpenoids in *A. occidentale* leaves, supporting their potential functional and pharmacological relevance (Bayuwana *et al.*, 2020). Variations in the phytochemical composition of *A. occidentale* have also been associated with abiotic factors, including soil characteristics and geographical location, indicating that environmental conditions can substantially influence its secondary metabolism.

The combined interpretation of the phytochemical and proximate profiles of *A. Occidentale* leaves is therefore particularly valuable because it provides information on both their nutritional characteristics and potential biological functionality. Differences between young and mature leaves may reflect developmental changes in photosynthetic activity, nutrient allocation, cellular structure and the accumulation of structural and secondary metabolites. Consequently, the higher or lower abundance of particular phytochemicals or proximate constituents in young versus mature leaves may represent physiological adaptation rather than random variation. However, the presence or abundance of phytochemicals should not be interpreted as direct evidence of therapeutic efficacy, since biological effects depend on the identity, concentration, bioavailability and interactions of individual compounds and should ideally be confirmed through complementary antioxidant, antimicrobial, pharmacological and toxicological investigations. The documented phytochemical richness of *A. Occidentale* leaves nevertheless supports their continued investigation as a potential source of nutritionally relevant and biologically active compounds (Oloruntola, 2021).

Conclusion

The findings of this study demonstrate that developmental stage significantly influences the proximate and phytochemical composition of *Anacardium occidentale* leaves. Mature leaves exhibited higher levels of protein, ash, crude fibre, and carbohydrate, as well as greater concentrations of phenolics, flavonoids, tannins, saponins, alkaloids, glycosides, terpenoids, and reducing sugars, indicating their greater overall nutritional and phytochemical richness. Conversely, sprout leaves showed higher moisture content and uniquely contained steroids, anthraquinones, and cardiac glycosides, suggesting that certain specialized metabolites are more prominent during early leaf development. The presence of diverse bioactive constituents in both developmental stages highlights the potential of *A. occidentale* leaves as valuable sources of nutritional and biologically active compounds. Overall, mature leaves appear more promising for applications requiring higher nutritional and major phytochemical content, while sprout leaves may be particularly valuable for compounds that were detected exclusively at the early developmental stage.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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