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A Comparative Study on the Phytochemical Constituents of Selected Plant Species in Relation to Their Leaf Maturity Stages

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Abstract

Secondary metabolites are essential in the therapeutic value of medicinal plants and their amounts significantly vary depending on the environment and their developmental stage. This comparative study aimed at understanding how the maturity of the leaves affects the quantitative phytochemical profile of eight medicinal plant species namely *Syzygium cumini*, *Calotropis procera*, *Tamarindus indicus*, *Parkia biglobosa*, *Mangifera indica*, *Eucalyptus camaldulensis*, *Azadirachta indica* and *Psidium guajava*. The phytochemicals analysed were Total Phenolic Content (TPC), Total Flavonoid Content (TFC), saponins, and alkaloids, in three different stages of leaf maturity (young, matured and senescent). Standardised extraction procedures were used and colourimetric and gravimetric assays were used to quantify. The analysis showed that the content of TPC and TFC had the same ontogenetic pattern across all eight species (matured leaves > young leaves > senescent leaves), and that the content in matured leaves was always the highest and significantly different ($P < 0.05$) from the other leaves. Nitrogenous compound and total alkaloid content showed high level of species-specific variation. The matured leaves contained maximum amount of alkaloids in six species (*T. indicus*, *P. biglobosa*, *M. indica*, *E. camaldulensis*, *S. cumini*, *A. indica*) while the senescent leaves contained maximum amount of alkaloids in *C. procera* and *P. guajava*, which corresponds to specialized nitrogen remobilization strategies. The saponin content was generally highest in young leaves ($P < 0.05$), as it is in the initial tissue defense, except in *A. indica* which reached its peak at maturity. It was observed that these results highlight the importance of maturity level of leaves as an important criterion to consider when harvesting medicinal plants for phytochemical production, therefore the need to establish uniform and standardized harvesting procedure based on the morphology of the plants to ensure phytochemical consistency and efficacy.

Introduction

Plant secondary metabolites are organic compounds that can be divided into three main groups: phenolic compounds, terpenes and steroids, and nitrogenous compounds according to their biosynthetic origin (Reshi et al., 2023). These molecules are not directly linked to primary growth but are key to plant survival and physiological processes such as stress adaptation and protection from herbivores and pathogens (Divekar et al., 2022). These specialized metabolites have found increasing importance in the pharmaceutical, food, and dye industry, leading to the need for efficient and reliable methods for their procurement and quality control. These specialized metabolites have been seen to play an increasing role in the pharmaceutical (Yeshi et al., 2022), food (Petropoulos 2023), and dye (Ozyigit et al., 2023) industries, which necessitates efficient and reliable methods to procure these specialized metabolites and to conduct quality control.

The plant's antioxidant pathway is regulated, among others, by phenolic compounds, which are produced in a significant part by the phenylpropanoid pathway, and include important compounds such as caffeic acid and gallic acid (Gomez-Espinoza et al., 2025). The flavonoids are one of the important sub-groups of phenolics which have well documented anti-inflammatory, anti-allergic and anti-cancer properties and are also extremely therapeutically significant (Zawawi et al., 2025). It has been reported that high concentrations of these compounds were associated with the ability of the plant to cope with the oxidative stress and external threat at its most metabolic phase (Shomali et al., 2022).

The basic nitrogen containing chemical compounds, which are formed mainly from amino acid precursors are called as alkaloids (Bhambhani et al., 2021). They are known for their high biological activity and have a wide range of medicinal properties, such as anti-proliferative effects (Tian et al., 2017), anti-plasmodial activity (Diaz et al., 2022), and analgesic activity (Wang et al., 2022). For example, some alkaloids present in *Calotropis procera* have been shown to have a strong antiproliferation activity (Naser et al., 2019). Saponins, a group of triterpenoid glycosides (Rashad 2025), are mainly defensive/antinutrient compounds of plants that effect palatability as well as act as toxic agents against herbivores (Shakeel et al., 2025). The pathways of their biosynthesis are complex, with the usual route being the mevalonate or MEP pathway (Chen et al., 2022), indicating that they require a significant metabolic investment.

The selections of plant species used in this comparative study (*Syzygium cumini*, *Calotropis procera*, *Tamarindus indicus*, *Parkia biglobosa*, *Mangifera indica*, *Eucalyptus camaldulensis*, *Azadirachta indica*, and *Psidium guajava*) cover varieties of phytochemistry and traditional uses that would create an adequate base for the ontogenetic variability assessment. Consequently, the leaves of *Syzygium cumini* are used to treat diabetes and diarrhea, and provide high levels of gallic acid, tannin, and flavonoids (Sohail et al., 2025). *Calotropis procera* has a number of therapeutic potentials including anti-fungal, anti-inflammatory, anti-diabetic and anti-leishmania (Singh et al., 2024). The leaves of both *Tamarindus indicus* and *Parkia biglobosa* have been documented with anti-inflammatory and anti-oxidative properties, which are related to their content of tannins and flavonoids such as kaempferol and epicatechin (Akter et al., 2023; Komolafe et al., 2024). These choices span from tannin-rich *T. indicus* to limonoid and saponin-rich *Azadirachta indica*, giving weight to the inquiry into the effects of different metabolic strategies on developmental cues (Nagini et al., 2024; Sami et al., 2018).

It has been established that concentrations of secondary metabolites depend on the age of the plant organ, as well as environmental conditions

(Aghaei et al., 2022; Zhao et al., 2023). However, a detailed multi-species mapping of the comparative accumulation kinetics of the major phytochemical classes (phenolics, flavonoids, alkaloids, and saponins) through the entire leaf lifecycle (young, matured, senescent) is still limited. This research aimed to fill this knowledge gap. It was hypothesized that carbon-based metabolites (TPC/TFC) would show a more universal peak associated with the peak of photosynthetic activity (maturity) under the assumption that they are metabolized under a common resource management and defense strategy, while nitrogenous (Alkaloids) and complex triterpenoids (Saponins) would demonstrate a higher degree of species-specific variation.

Materials and Methods

Plant Material

Three maturity stages were defined using a stringent set of morphological criteria. Immature leaves were characterized as those that showed active cell expansions, low mechanical integrity and light green color. Matured leaves were fully expanded, structurally strong, deep green, and with maximum photosynthetic potential. Senescent leaves displayed apparent senescence symptoms such as chlorosis (yellowing/browning), which are a sign of programmed cell death and chlorophyll degradation (Han et al., 2024). Samples were collected and dried and ground to fine powder for phytochemical analysis.

Extraction Procedure and Quantification

Optimized solvent extraction systems, including 80% methanol/water, were used to prepare plant extracts for compounds that were polar phenolics, semi-polar alkaloids and saponins. For the best quantitative recoveries of various chemical classes, sequential extraction methods were used.

Determination of Total Phenolic Content (TPC)

The standardised Folin-Ciocalteu (FC) colorimetric assay was used to quantify the TPC. The method is based on an electron-transfer reaction where antioxidant species in the extract transfer the electron to the FC reagent (Perez et al., 2023). The dilution was done by mixing diluted extracts with the FC reagent followed by neutralization by 7.5% anhydrous sodium carbonate solution which provides the basic condition required for the redox reaction to take place. After 2 h incubation at RT, the absorbance of the mixture was measured at 765 nm (Zhou et al., 2020). Gallic acid was used to prepare the calibration curve and the results were presented as milligrams of Gallic Acid Equivalent per gram dry matter (mg GAE/g DW).

Determination of Total Flavonoid Content (TFC)

The colorimetric assay method of Aluminum Chloride (AlCl3) was used to quantify TFC. The method is based on the ability of AlCl3 to form stable complexes with the C-4 keto group and either the C-3 hydroxyl group or the C-5 hydroxyl group of certain flavonoids group like flavones and flavonols (Bhaigyabati et al., 2014). Quercetin was used as the standard compound and spectrophotometrically measured. The use of a blank extract (without AlCl3) to compensate for the natural yellow or brown colouration that may occur in extracts from mature or senesced plants to minimize the potential limitations of colorimetric assays (Sultana et al., 2024). The results were calculated using standard curve and dilution factor (mg Quercetin Equivalents/g DW).

Quantification of Total Alkaloids and Saponins

The total alkaloid content was determined by the spectrophotometric method of Bromocresol Green (BCG). A small portion of the acidic

alkaloid extract (or Atropine standard solution) was added to a separating funnel to which was added a quantity of a pH 4.7 phosphate buffer and a quantity of BCG solution. The mixture was shaken vigorously with chloroform to remove the yellow BCG-alkaloid ion-pair complex between the protonated alkaloid and the dye ion. The chloroform separates and was collected and repeated with further chloroform until complete extraction was achieved. The combined chloroform extracts were then made up to a standard volume and the absorbance of the complex was spectrophotometrically recorded at 470 nm against the reagent blank, the total alkaloid content was determined from the standard curve and expressed as (mg AE/g DW). The overall saponin content was estimated by using a modified Vanillin Sulfuric Acid colorimetric method and Diosgenin as the standard reference for construction of the calibration curve. In brief, 0.25 mL of sample extract (after evaporation of the extraction solvent to reduce interference) or the standard solutions were added sequentially to 0.25 mL of 8% vanillin-ethanol solution and 2.50 mL of 72% sulfuric acid-water solution, all of which were added in an ice bath. The mixtures were then placed at 60oC for 15 minutes to develop color, were cooled in an ice bath for 5 minutes to stop the reaction and absorbance was measured at 544 nm against a reagent blank, reported in milligrams of the standard equivalent per gram of dry sample (mg DE/g DW) (de Aguiar et al 2024).

Statistical Analysis

Each of the quantitative data were analyzed by Analysis of Variance (ANOVA) for statistical significance of the main factors (Plant Species and Leaf Maturity Stage). Post hoc analysis was conducted to determine if significant differences exist between the three maturity stages for each species.

Results and Discussion

Results

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The difference in the TPC and TFC of young, matured and senescent plants is quite significant ($P< 0.05$). The overall pattern of both compound classes was maintained for all 8 plant species studied. As shown in Figure 1 and 2.

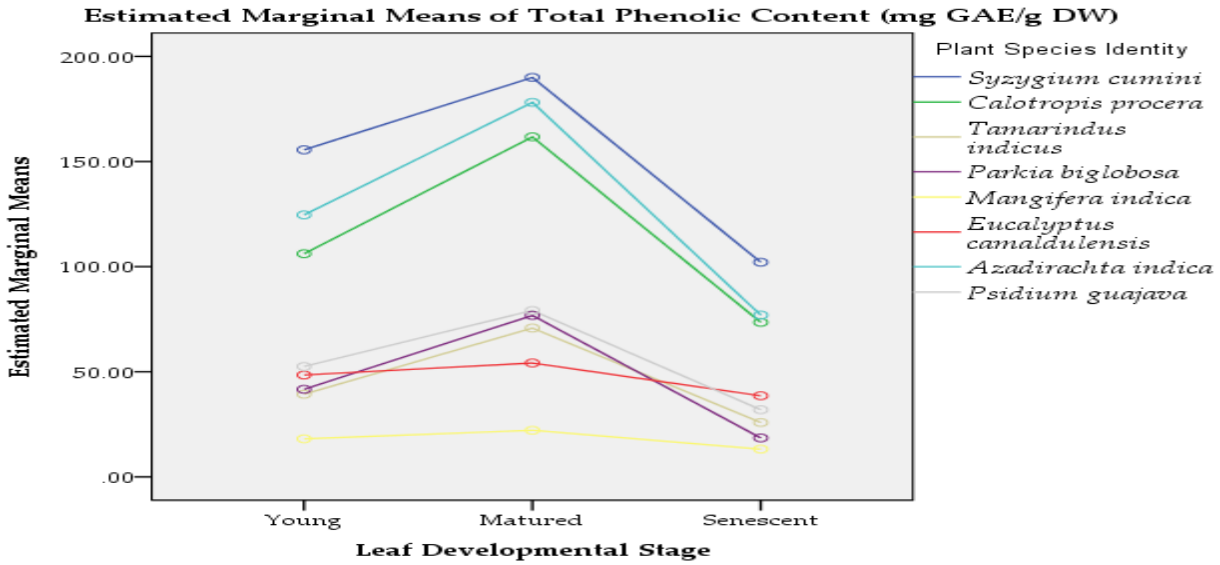

Figure 1: Total Phenolic Content (TPC) of Selected Plant Species in Relation to Their Leaf Maturity Stages

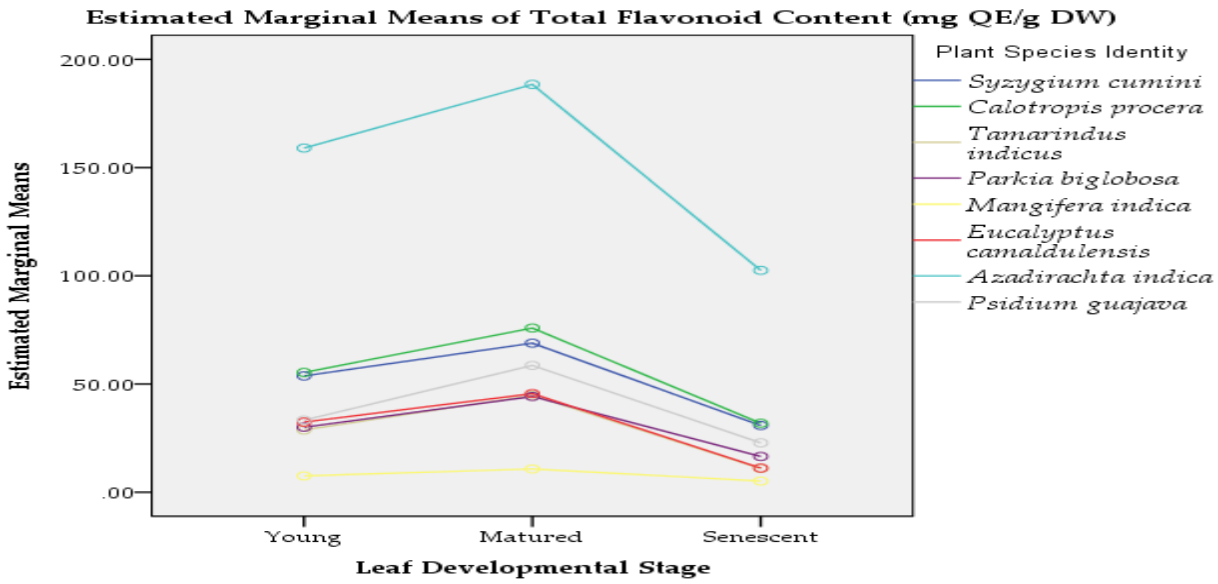

Figure 2: Total Flavonoid Content (TFC) of Selected Plant Species in Relation to Their Leaf Maturity Stages

TPC and TFC always showed a gradient of concentration from mature leaves to young leaves and young leaves to senescent leaves, with the mature leaves being significantly higher in concentration than the young leaves, and the young leaves significantly higher in concentration than the senescent leaves ($P < 0.05$). This has been observed in previous research, which indicated that the level of T.P.C was higher in mature leaves than in young leaves (Kittibunchakul et al., 2022). The mean TPC was found to be about 40-60% lower in the senescent leaves than in mature leaves, suggesting significant net loss of these carbon-based compounds in the last developmental stage. The regularity of this relationship implies that the age of leaf functional maturity could be the determining factor for the maximum investment into the constitutive antioxidant defense.

Total Alkaloid Content

The ontogenic variability of the alkaloid content was the highest (Figure 3) and was shown to be highly significant ($P < 0.05$) from the ANOVA Species x Maturity interaction effect. The time course of alkaloid accumulation was divided into two separate metabolic groups:

1. **Matured Leaf Peak (Group A):** Five plant species, namely *Tamarindus indicus*, *Parkia biglobosa*, *Mangifera indica*, *Eucalyptus camaldulensis* and *Syzygium cumini* exhibited the highest alkaloids content at their matured stage (Group A). For *S. cumini*, the content of alkaloid was found to be minimum at young leaf stage, indicating that significant synthesis of alkaloids starts mainly after the cessation of active cell expansion and increases during functional phase.

2. **Senescent Leaf Peak (Group B):** *Calotropis procera* and *Psidium guajava* showed a significant metabolic change which became the highest concentration of alkaloids at senescent leaf stage. This is a pattern opposite to that seen for most secondary metabolites, and suggests a very specific nitrogen partitioning strategy in these species.

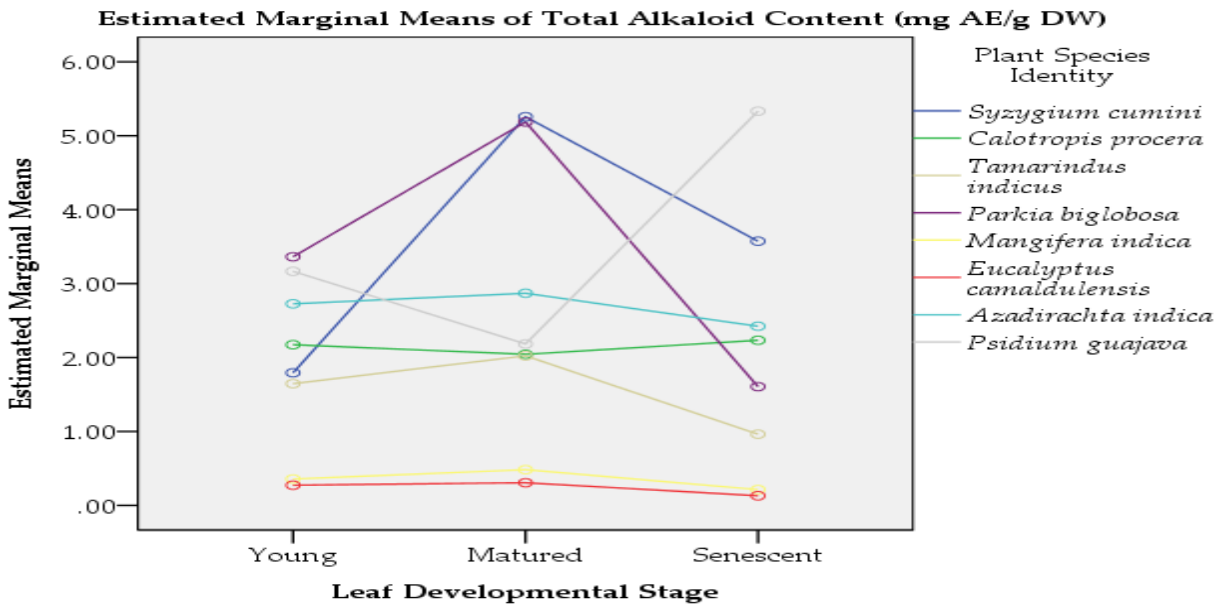

Figure 3: Total Alkaloid Content of Selected Plant Species in Relation to Their Leaf Maturity Stages

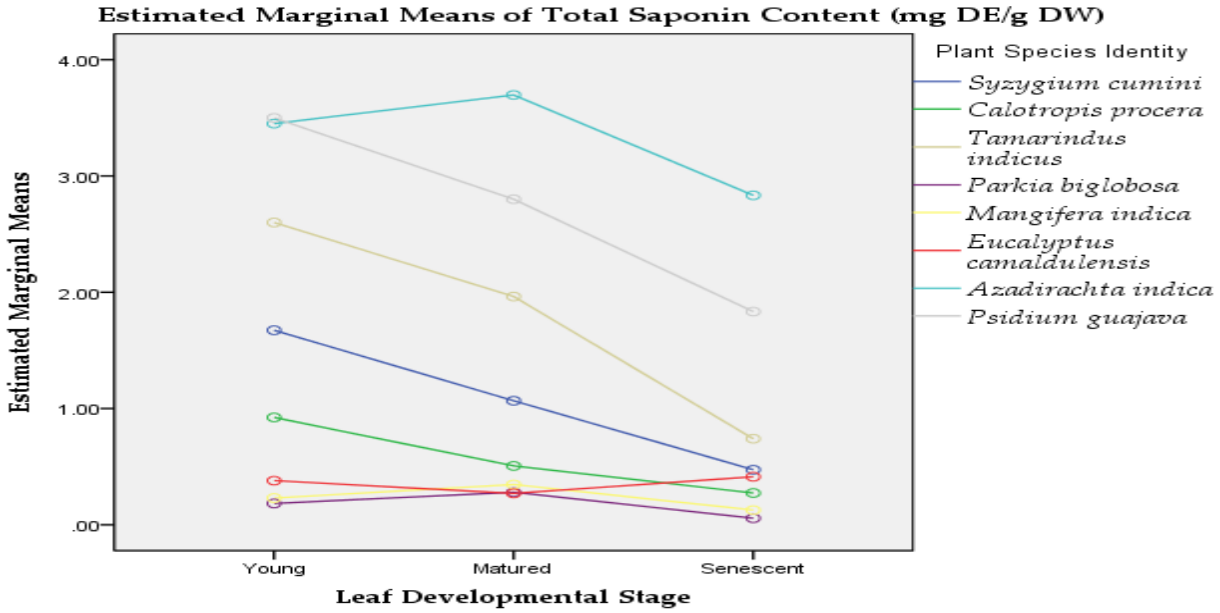

Figure 4: Total Saponin Content of Selected Plant Species in Relation to Their Leaf Maturity Stages

Total Saponin Content

The quantification of saponin content showed a general pattern that is correlated with early defense provisioning. Saponin content was the highest in young leaves and the lowest in senescent leaves as presented in Figure 4 for 7 of the 8 species. This indicates that metabolically, immediate chemical protection of vulnerable actively growing foliage is more important.

One exception was found in *Azadirachta indica*. In the present species, the matured leaves contained the maximum amount of saponin and senescent

leaves contained the minimum amount of saponin ($P<0.05$). Thus, this anomaly seems to reflect a connection between the synthesis of saponin and the complex triterpenoid pathway involved in the synthesis of the specialized defense chemicals (limonoids) of *A. indica* such as azadirachtin, with maximum production of triterpenoids occurring only when the leaves are fully mature.

Comparative study of phytochemical constituents of selected plant species with their maturity stages of leaves is presented in Table 1. All the quantified phytochemicals were found to be significantly different ($P < 0.05$) among the leaf maturity stages of each plant species (except slight difference in *C. procera* and *E. camaldulensis* with *A. indica*).

Discussion

Carbon Allocation and Peak TPC/TFC at Maturity

It is found universally that TPC and TFC levels are maximum in the matured leaves for all eight species, which testifies to a general plant metabolic strategy. The mature leaves are the site of maximum photosynthesis ability and have the highest capacity to bind carbon resources that are important precursors for the production of carbon rich phenolic chemicals (Kittibunchakul et al., 2022). These compounds have two functions: as powerful soluble antioxidants and as part of the structure (lignin and tannins). The regular accumulation at this stage is the greatest and most typical investment in constitutive defense that a leaf must make to keep itself safe while it serves its maximum functional role.

TPC and TFC were also found to decrease significantly during senescence (40-60%), as a result of the metabolic shift from defense maintenance to nutrient recovery. The process of senescence is genetically controlled and is used to mobilize mobile resources (such as N and P) prior to abscission of the leaves (Liu et al., 2008). The mobile phenolic fractions are also oxidized by enzymes or by other reactions, resulting in a considerable loss of total antioxidant capacity of the senescing tissue (Han et al., 2024), while chlorophyll is degraded rather quickly.

Total Alkaloid Content

The significant amount of alkaloids in senescent leaves of *Calotropis procera* and *Psidium guajava* (Group B) is an exception to the recovery of resources and is of great interest for specialized nitrogen management.

Senescence triggers massive protein degradation, which leads to a burst of free amino acids (mobile nitrogen) to be exported to developing storage tissues such as seeds or roots (Yuan et al., 2023). When the flow of these amino acids is blocked, retarded or these nitrogenous precursors are actively utilized in certain alkaloid biosynthetic pathways, the outcome is the buildup of nitrogenous substances. Alkaloids serve as chemical sinks to immobilize nitrogen not being exported within the leaf tissue (Yuan et al., 2023). This late stage synthesis may be a defense mechanism in which highly deterrent compounds (such as alkaloids in *C. procera* (Naser et al., 2019)) build up to protect the nitrogen rich senescing tissue from late stage herbivores. It shows that in these species, the accumulation of nitrogenous metabolites does not solely depend on defense kinetics, but rather, is closely related to nitrogen economy and remobilization efficiency.

Total Saponin Content

In the majority of species, saponins are more abundant in young leaves which are more susceptible to attack, thus providing further evidence of the ecological rule that the most vulnerable tissues in most species must be protected first. Young leaves are metabolically expensive and expensive to produce because they have a high nutritional value and low structural defenses, and are therefore highly valued by herbivores, which is why they are

abundantly allocated resources to make strong chemical defenses, such as saponins (Heuze et al., 2015).

The saponin peak is delayed in *Azadirachta indica* and is at the highest level when the leaves are matured, which implies that it has a complex internal metabolic regulation. *A. indica* is renowned for producing limonoids (a type of triterpenoid, like azadirachtin), which are highly effective natural pesticides (Rao & Kumar 2023). Triterpenoids such as saponins could be synthesized by similar enzymatic steps as in the complex limonoid pathway. Potential activity and maximum output of this specialized defense system might only be attained during the mature leaf stage, therefore the co-accumulation of limonoids and saponins at the mature stage.

Table 1: Comparative Ontogenetic Accumulation Patterns

		TPC (mg GAE/g)	TFC(mg QE/g DW)	Alkaloid (mg AE/g DW)	Saponin (mg DE/g DW)
S. cumini	Young	155.56 ± 4.23b	53.73 ± 1.65b	1.79 ± 0.11a	1.67 ± 0.06c
	Matured	190.01 ± 2.12c	68.89 ± 0.34c	5.26 ± 0.16c	1.07 ± 0.15b
	Senescent	102.11 ± 1.01a	30.83 ± 1.12a	3.57 ± 0.36b	0.47 ± 0.08a
C. procera	Young	106.13 ± 5.88b	55.34 ± 2.76b	2.17 ± 0.02b	0.92 ± 0.04c
	Matured	161.73 ± 6.94c	75.87 ± 4.68c	2.04 ± 0.05a	0.51 ± 0.02b
	Senescent	73.50 ± 3.75a	31.86 ± 0.91a	2.23 ± 0.01b	0.27 ± 0.05a
T. indicus	Young	39.30 ± 0.53b	28.77 ± 1.03b	1.65 ± 0.05b	2.60 ± 0.20c
	Matured	70.80 ± 1.25c	44.77 ± 0.91c	2.02 ± 0.07c	1.96 ± 0.12b
	Senescent	25.83 ± 2.91a	10.93 ± 0.75a	0.96 ± 0.12a	0.74 ± 0.06a
P. biglobosa	Young	41.65 ± 1.45b	30.04 ± 1.34b	3.36 ± 0.24b	0.28 ± 0.01c
	Matured	76.80 ± 1.43c	44.26 ± 1.89c	5.19 ± 0.10c	0.18 ± 0.01b
	Senescent	18.51 ± 1.39a	16.51 ± 1.76a	1.61 ± 0.25a	0.06 ± 0.03a
M. indica	Young	18.10 ± 0.44b	7.53 ± 0.25b	0.36 ± 0.04b	0.35 ± 0.03c
	Matured	22.17 ± 0.81c	10.77 ± 0.51c	0.48 ± 0.01c	0.23 ± 0.03b
	Senescent	13.20 ± 1.08a	5.17 ± 0.06a	0.21 ± 0.01a	0.13 ± 0.02a
E. camaldulensis	Young	48.47 ± 1.00b	32.40 ± 0.35b	0.27 ± 0.01b	0.38 ± 0.01c
	Matured	54.17 ± 1.99c	45.57 ± 0.25c	0.31 ± 0.01b	0.27 ± 0.03b
	Senescent	38.57 ± 0.29a	11.17 ± 0.95a	0.13 ± 0.03a	0.11 ± 0.02a
A. indica	Young	124.60 ± 2.36b	159.00 ± 2.04b	2.73 ± 0.02b	3.45 ± 0.05b
	Matured	178.13 ± 1.60c	188.43 ± 0.83c	2.87 ± 0.01b	3.70 ± 0.06c
	Senescent	76.93 ± 0.97a	102.50 ± 0.52a	2.42 ± 0.12a	2.83 ± 0.06a
P. guajava	Young	52.51 ± 1.63b	33.30 ± 1.82b	3.17 ± 0.25b	3.50 ± 0.26c
	Matured	79.17 ± 0.50c	58.60 ± 1.15c	2.18 ± 0.10a	2.80 ± 0.10b
	Senescent	31.93 ± 0.96a	22.80 ± 1.54a	5.33 ± 0.40c	1.83 ± 0.06a

Different small letters indicate significant differences ($P < 0.05$) among leaf maturity stages of each plant species.

Critical Implications for Quality Control and Pharmaceutical Sourcing

The observed differences in phytochemical composition by maturity stage indicate the need for uniformity in harvesting methods. When the application requires a general antioxidant, anti-inflammatory or structural compound (TPC/TFC), the robust matured leaf stage is the default harvest stage, yielding maximum and consistent harvest. The removal of senescent leaves, irrespective of the species, has the risk of substantial reduction in these significant components of carbon (Ghimire et al., 2021).

But the irregular accumulation of alkaloids and saponins calls for careful consideration that is guided by the target therapy. When the ultimate objective is to maximise the yield of a particular alkaloid in senescence (e.g., *C. procera* or *P. guajava*), the pharmaceutical manufacturers should deliberately choose the senescent stage with the awareness of the inevitable compromise of lower yields of TPC/TFC.

The findings of this study highlight the importance of introducing phytomorphological criteria instead of chronological timing for the identification of the quality of raw materials, from a regulatory point of view. The addition of morphological standards in Good Agricultural and Collection Practices (GACP) ensure that batches are collected at the moment of maximum concentration of therapeutic markers, guaranteeing batch-to-batch consistency and the pharmacological effectiveness (Ryu *et al*., 2023).

Conclusion

The present comparative study has been proved that there is a significant and profound effect of leaf maturity stage on phytochemical profile of medicinal plants. The carbon-based defenses (TPC and TFC) increases according to conserved, ontogenetically determined peak. Nitrogenous and complex terpenoid compounds, on the other hand, are highly species-specific, indicative of sophisticated metabolic prioritization strategies related to the complexities of defense kinetics and resource remobilization efficiency. It is noteworthy that, in each of the eight species, the matured stage is shown to be best for high yields of TPC and TFC, whereas there are exceptions for species such as *C. procera* and *P. guajava*, in which the stage of senescence is optimal.

Harvesting procedures need to be harmonized to ensure efficacy and consistency of pharmaceutical ingredients from plants, by using precise definitions of morphological maturity. For general therapeutic uses which include antioxidant and anti-inflammatory activity, the morphologically defined matured leaf stage should be adopted as standard. The criteria for harvesting of young or senescent stages must be scientifically substantiated, and made explicit quality specifications related to defined therapeutic targets, and must be based on quantitative data presented here.

Lastly, controlled environmental research is required to measure the effect of the intrinsic ontogenetic schedule (e.g. drought, temperature change) on the metabolic output, since environmental factors can greatly affect the metabolic output.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: study design: Idris, A; data collection: Abigail, R., Maryam, S. M., Hasina, S. A., Khadija,

B. M., Abdussamas, A., Adam, A. A., Halimat, S. O., and Favour, C. D.; analysis and interpretation of results: Idris, A, Mohammad. M; draft manuscript preparation: Idris A, Mohammad. M. All authors reviewed the results and approved the final version of the manuscript.

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