

Comparative Phytochemical Analysis of Mistletoe-Infested and Non-Infested *Terminalia Catappa* L. Leaves

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ABSTRACT

Phytochemicals are vital bioactive compounds that contribute to plant defence mechanisms and human health. This study investigated the impact of mistletoe (*Loranthus sp.*) infestation on the phytochemical composition of *Terminalia catappa* L. (Combretaceae) leaves a medicinally and ecologically significant tropical species widely used in ethnomedicine. Fresh leaves were collected from healthy (HTL), branch-infested (INF-B-L), and generally infested (INF-G-L) *T. catappa* trees within Ahmadu Bello University, Zaria, Nigeria. Standard qualitative and quantitative phytochemical analyses were performed on methanolic extracts using spectrophotometric assays for alkaloids, flavonoids, saponins, phenols, and tannins. Data were statistically analysed using one-way ANOVA and Tukey's post-hoc test at a 95% confidence level ($p < 0.05$). Results revealed significant ($p < 0.05$) reductions in alkaloids (1.23 ± 0.01 mg/g \rightarrow 1.06 ± 0.01 mg/g), flavonoids (9.62 ± 0.02 mg/g \rightarrow 8.88 ± 0.03 mg/g), phenols (6.52 ± 0.02 mg/g \rightarrow 6.19 ± 0.02 mg/g), and tannins (1.84 ± 0.01 mg/g \rightarrow 1.70 ± 0.01 mg/g) from healthy to infested leaves, respectively, while saponins increased slightly ($1.22 \pm 0.01 \rightarrow 1.26 \pm 0.01$ mg/g). Anthraquinones were absent in all samples. These findings show that mistletoe infestation alters the bioactive compounds of *T. catappa* leaves and highlight the importance of regular monitoring and control of parasitic plants to maintain the therapeutic integrity of economically and ethnobotanically valuable species.

Keywords: *Terminalia catappa*, mistletoe infestation, phytochemical composition, secondary metabolites, host-parasite interaction

1.0 INTRODUCTION

Terminalia catappa L. (Combretaceae), commonly known as the Indian or tropical almond, is a multipurpose tree widely distributed across tropical and subtropical regions. Native to Southeast Asia and the Pacific Islands, it has become naturalized across Africa and the Americas due to its high ecological adaptability and ornamental value (Suresh *et al.*, 2025). The species is well recognized in ethnomedicine for its broad spectrum of therapeutic activities, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, and anticancer properties (Mwangi *et al.*, 2024; Pranjali and Ravi, 2020). These pharmacological benefits are attributed to its rich composition of secondary metabolites particularly phenolics, flavonoids, and tannins which are concentrated in the leaves and fruit pericarp. Medicinal plants synthesize a wide range of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, and phenolic compounds that serve in plant defense, animal growth stimulant, essential ecological and pharmacological roles (Zeeshan *et al.*, 2022; Zandavar and Afshari 2023; Svetlana *et al.*, 2024; Abdullllahi and Hamza, 2020).

Phytochemicals in plants are biosynthesized as part of complex metabolic networks that respond dynamically to both abiotic and biotic stressors. Among the latter, mistletoe infestation is one of the most ecologically significant yet understudied interactions influencing host plant chemistry. Mistletoes are obligate aerial hemiparasitic plants belonging primarily to the Loranthaceae family. They attach to host branches via specialized structures called haustoria, which penetrate the xylem to extract water and nutrients while maintaining their own photosynthetic activity (Muche *et al.*, 2020). This nutrient siphoning often induces physiological stress in the host, potentially impairing

growth, photosynthesis, and metabolic stability (Jones and Taylor, 2019).

Although mistletoe does not usually kill its host outright, prolonged infestation can result in altered biochemical processes, particularly in secondary metabolism, as the host reallocates resources to defence and survival (Chen *et al.*, 2022). The interaction may stimulate the synthesis of certain stress-related compounds like saponins or terpenoids, while simultaneously reducing the accumulation of others such as phenolics and flavonoids that depend on carbohydrate and nitrogen balance (Fernanda *et al.*, 2019). As these metabolites are often directly responsible for medicinal and antioxidant functions, changes in their concentration could have implications for the pharmacological value of the plant (Isikhuemen *et al.*, 2020).

Despite the medicinal importance of *Terminalia catappa*, research on how parasitic stress especially from mistletoe infestation affects its phytochemical composition remains scarce. However, there is limited quantitative data specific to *T. catappa*, leaving a crucial gap in understanding the chemical and therapeutic consequences of mistletoe infestation on this species. Therefore, this study aims to provide a comparative assessment of the phytochemical composition of mistletoe-infested and non-infested *Terminalia catappa* leaves. Specifically, it evaluates both qualitative and quantitative variations in major secondary metabolites to determine the biochemical effects of mistletoe parasitism.

2.0 MATERIALS AND METHODS

i. Study Area

This study was conducted at Ahmadu Bello University (ABU), Zaria, Kaduna State, Nigeria, located within the Northern Guinea Savannah ecological zone. The area is

characterized by distinct wet and dry seasons, with annual rainfall ranging between 1,000 and 1,200 mm and an average temperature of 18–34°C.

ii. Plant Collection and Identification

Fresh and mature leaves of *Terminalia catappa* were collected from both healthy and mistletoe-infested trees within the ABU Zaria campus at Dangote Hostel Phase II (11.13672° N, 7.63879° E), Centre of Excellence Complex (11° 08' 26.00" N, 007° 39' 05" E), in front of the Biological Complex (11° 09' 03.90" N, 007° 39' 09.30" E, and behind the Department of Microbiology 11° 09' 06.70" N, 007° 39' 08.40" E, and grouped into three categories: HTL (healthy leaves), INF-B-L (leaves from branches attached to mistletoe), and INF-G-L (leaves from mistletoe-infested trees) with species identification and authentication carried out at the Herbarium Unit, Department of Botany, Ahmadu Bello University, Zaria.

iii. Preparation of Plant Materials

The collected leaves samples were air-dried under shade at ambient temperature (25–28°C) for two weeks to prevent degradation of heat-sensitive compounds. The dried materials were then ground into a fine powder using a

Table 1. Phytochemical qualitative assays conducted

| Phytochemical | Test Performed | Positive Indication |
|----------------|--|---|
| Alkaloids | Mayer's Test – Extract + Mayer's reagent | Cream precipitate |
| Carbohydrates | Molisch's Test – Extract + Molisch reagent + conc. H ₂ SO ₄ | Violet ring at interface |
| Saponins | Frothing Test – Extract shaken with water, then olive oil added | Stable persistent foam |
| Flavonoids | Alkaline Reagent Test – Extract + NaOH → yellow; disappears with HCl | Yellow coloration fades |
| Phenols | Lead Acetate Test – Extract + Pb(CH ₃ COO) ₂ | Yellow precipitate |
| Tannins | Ferric Chloride Test – Extract + FeCl ₃ | Blue-green or blue-black coloration |
| Terpenoids | Liebermann–Burchard Test – Extract + acetic anhydride + conc. H ₂ SO ₄ | Reddish-violet → greenish-blue coloration |
| Steroids | Salkowski's Test – Extract + conc. H ₂ SO ₄ | Reddish-brown coloration |
| Anthraquinones | Borntrager's Test – Extract + benzene, then ammonia solution | Pink/red coloration in ammoniacal layer |

All reagents were prepared freshly, and the visual colour changes were immediately recorded to avoid degradation of unstable compounds.

vi. Quantitative Phytochemical Analysis

Quantitative determinations were carried out using UV–Visible spectrophotometry for five major classes of secondary metabolites: alkaloids, flavonoids, phenols, tannins, and saponins. Each assay was performed in triplicate, and results were expressed in mg of standard equivalent per gram of extract.

a. Total Phenolic Content (TPC)

Phenolic content was determined using the Folin–Ciocalteu colorimetric method (Ainsworth and Gillespie, 2007).

b. Total Flavonoid Content (TFC)

Flavonoids were quantified using the Aluminum Chloride (AlCl₃) colorimetric method (Chang *et al.*, 2002).

c. Total Alkaloid Content (TAC)

Alkaloids were determined following the Bromocresol Green (BCG) spectrophotometric method (Shamsa *et al.*, 2008).

d. Total Tannin Content

Tannin concentration was estimated using the Folin–Ciocalteu method as described by Marinova *et al.* (2005).

e. Total Saponin Content

Saponin quantification followed the Vanillin–Sulphuric Acid method (Makkar *et al.*, 2007).

vii. Data Analysis

All assays were conducted in triplicate, and results were expressed as mean ± standard error of mean (SEM); data were analysed using SPSS version 20.0, employing one-way ANOVA to test for differences in phytochemical concentrations among HTL, INF-B-L, and INF-G-L samples, followed by Tukey's HSD post-hoc test for multiple comparisons at a significance level of $p < 0.05$.

sterilized mortar and pestle and stored in well-labelled airtight containers.

iv. Extraction of Phytochemicals

Fifty grams (50 g) of each powdered sample were extracted with 200 mL of methanol using the cold maceration method, following standard procedures described by Harborne (1998) and Sofowora (2008). The mixtures were intermittently agitated and allowed to stand for 72 hours to ensure maximum extraction. The resulting extracts were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated using a rotary evaporator at temperatures below 40°C to preserve thermolabile compounds (Trease and Evans, 2002). The crude extracts were then transferred into labelled glass bottles and stored at 4°C prior to qualitative and quantitative analyses.

v. Qualitative Phytochemical Screening

The methanolic extracts were subjected to standard phytochemical screening to identify the presence of major secondary metabolites. Each test was performed in triplicate, adopting procedures outlined by Harborne (1998), Trease and Evans (2002), and Sofowora (2008) with minor modifications.

3.0 RESULTS

i. Qualitative Phytochemical Composition of *Terminalia catappa* Leaves

The qualitative phytochemical screening of methanolic extracts from healthy (*HTL*), branch-infected (*INF-B-L*), and generally infected (*INF-G-L*) *Terminalia catappa* leaves revealed the presence of multiple classes of secondary metabolites (Table 1). Alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids, and terpenoids were all detected across all samples. However, anthraquinones were absent in every leaf category examined. These findings confirm that *T. catappa* synthesizes a broad range of bioactive phytochemicals regardless of infection status, though mistletoe parasitism may influence the degree of metabolite accumulation.

Table 2: Qualitative phytochemical constituents of healthy and mistletoe-infested *Terminalia*

Keys: + = Present; - = Absent, H-T-L = Healthy *Terminalia catappa* Leaves, INF-B-L = Infected Branch Leaves, INF-G-L = Infected General Leaves

ii. Quantitative Phytochemical Composition

Quantitative assessment of the methanolic extracts revealed statistically significant variations ($p < 0.05$) in the concentrations of major secondary metabolites among the three sample categories (Table 2). The general pattern showed a decrease in metabolite concentration from healthy to mistletoe-infested leaves, except for saponins, which exhibited a slight increase in generally infected leaves (*INF-G-L*).

| S/No | Phytochemical | Test | INF-B-L-001 | H-T-L-001 | INF-G-L-001 | INF-B-L-002 | H-T-L-002 | INF-G-L-002 |
|------|--------------------|---------------------|-------------|-----------|-------------|-------------|-----------|-------------|
| 1 | Alkaloids | Dragendorff | + | + | + | + | + | + |
| 2 | Cardiac Glycosides | Keller-Kilian | + | + | + | + | + | + |
| 3 | Saponins | Frothing | + | + | + | + | + | + |
| 4 | Phenolic compounds | Lead acetate | + | + | + | + | + | + |
| 5 | Tannins | Ferric chloride | + | + | + | + | + | + |
| 6 | Steroids | Salkowski | + | + | + | + | + | + |
| 7 | Carbohydrates | Molisch | + | + | + | + | + | + |
| 8 | Flavonoids | Shinoda | + | + | + | + | + | + |
| 9 | Terpenoids | Liebermann-Burchard | + | + | + | + | + | + |
| 10 | Anthraquinones | Bontrager's | - | - | - | - | - | - |

a. Alkaloids

The total alkaloid content of *T. catappa* leaves ranged from 1.06 ± 0.01 mg/g in *INF-G-L* to 1.23 ± 0.01 mg/g in *HTL*. The differences among all three categories were statistically significant ($p = 0.0002$). Healthy leaves exhibited the highest alkaloid concentration, while generally infected leaves had the lowest. This suggests that mistletoe infestation interferes with nitrogen metabolism and alkaloid biosynthesis in the host plant.

b. Flavonoids

Flavonoid concentrations were significantly affected by mistletoe infestation ($p < 0.0001$). Healthy leaves contained 9.62 ± 0.02 mg/g, branch-infected leaves 9.19 ± 0.06 mg/g, and generally infected leaves 8.88 ± 0.03 mg/g. The decreasing trend indicates that parasitic infection suppresses flavonoid biosynthesis, possibly through reduced photosynthetic efficiency and metabolic diversion caused by nutrient competition. Flavonoids are key antioxidants; therefore, their reduction in infected leaves implies diminished oxidative defence capacity.

c. Phenols

The total phenolic content followed a similar declining pattern, with values of 6.52 ± 0.02 mg/g (HTL), 5.93 ± 0.05 mg/g (INF-B-L), and 6.19 ± 0.02 mg/g (INF-G-L). The differences were significant ($p < 0.0001$). Although generally infected leaves exhibited a slightly higher phenolic content than branch-infected ones, both remained lower than the healthy control.

d. Tannins

Tannin concentration also showed a significant decline across the sample categories ($p < 0.0001$), with mean values of 1.84 ± 0.01 mg/g in HTL, 1.82 ± 0.01 mg/g in INF-B-L, and 1.70 ± 0.01 mg/g in INF-G-L. Tannins are essential defence metabolites with antimicrobial and wound-healing properties. Their reduction in infested leaves implies a compromised defence mechanism against pathogens and herbivores.

e. Saponins

In contrast to other metabolites, saponins showed a marginal but statistically significant ($p = 0.0046$) increase in infested leaves. Concentrations were 1.22 ± 0.01 mg/g (HTL), 1.18 ± 0.02 mg/g (INF-B-L), and 1.26 ± 0.01 mg/g (INF-G-L).

The elevated saponin level in generally infected leaves may reflect a stress-induced biochemical response aimed at enhancing host defence

Table 3: *Quantitative phytochemical composition (mean \pm SEM) of healthy and mistletoe infested Terminalia catappa leaves*

| Phytochemical | HTL (Healthy Leaves) | Tree INF-B-L (Infected Branch Leaves) | INF-G-L (Infected General Leaves) | P-value |
|---------------|----------------------|---------------------------------------|-----------------------------------|---------|
| Alkaloids | 1.23 ± 0.01^a | 1.13 ± 0.03^b | 1.06 ± 0.01^c | 0.0002 |
| Flavonoids | 9.62 ± 0.02^a | 9.19 ± 0.06^b | 8.88 ± 0.03^c | <0.0001 |
| Saponins | 1.22 ± 0.01^a | 1.18 ± 0.02^b | 1.26 ± 0.01^c | 0.0046 |
| Phenols | 6.52 ± 0.02^a | 5.93 ± 0.05^c | 6.19 ± 0.02^b | <0.0001 |
| Tannins | 1.84 ± 0.01^a | 1.82 ± 0.01^b | 1.70 ± 0.01^c | <0.0001 |

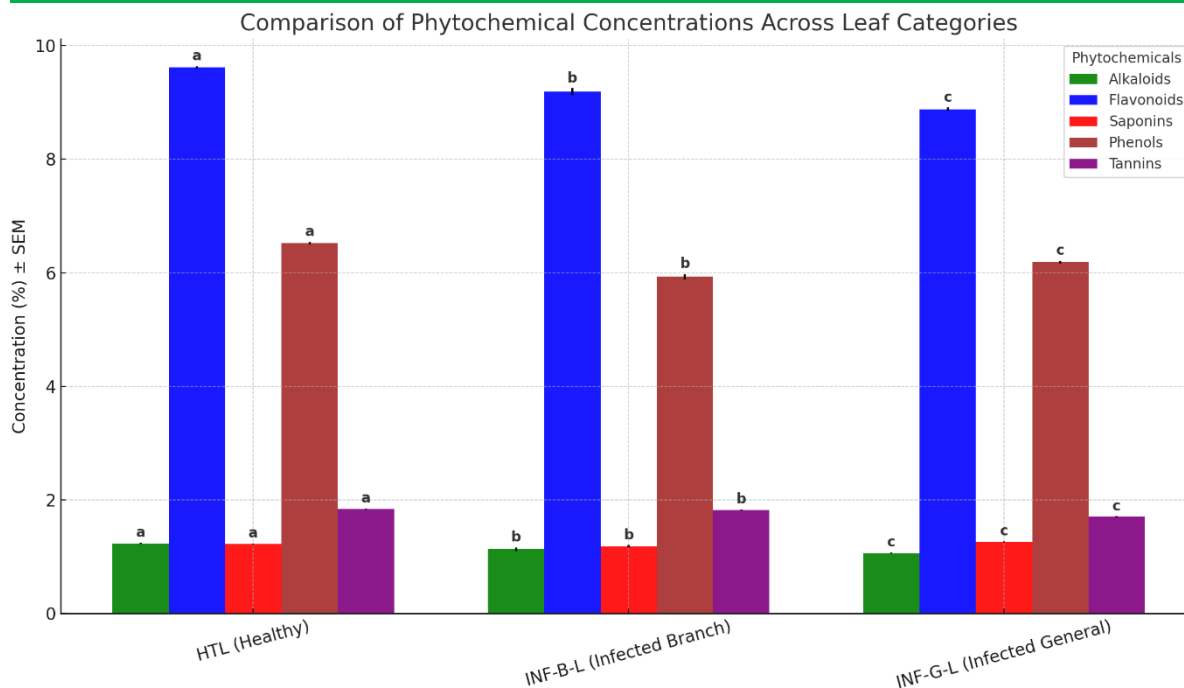


Figure 1: Comparative concentrations of major phytochemicals (alkaloids, flavonoids, phenols, tannins, and saponins) in healthy (HTL), branch-infected (INF-B-L), and generally infected (INF-G-L) *Terminalia catappa* leaves.

4.0 DISCUSSION

This study examined the comparative phytochemical composition of mistletoe-infested and non-infested *Terminalia catappa* leaves. The findings provide evidence that mistletoe parasitism exerts a measurable impact on the biosynthesis and accumulation of key secondary metabolites, including alkaloids, flavonoids, phenols, tannins, and saponins. The results revealed that infestation significantly reduced the concentrations of alkaloids, flavonoids, phenols, and tannins, while saponins exhibited a slight but notable increase. These changes demonstrate a likely biochemical imbalance in parasitized leaves, resulting from nutrient competition, oxidative stress, and altered carbon–nitrogen allocation induced by the mistletoe. Mistletoes are obligate aerial hemiparasites that attach to host trees via haustoria, specialized structures that penetrate the host's xylem tissues to draw water and mineral nutrients (Muche *et al.*, 2020). Although they retain chlorophyll and conduct photosynthesis, mistletoes depend heavily on their hosts for metabolic resources (Jones and Taylor, 2019). This parasitic relationship imposes physiological stress on the host plant, disrupting nutrient flow, photosynthetic efficiency, and growth (Chen *et al.*, 2022). The host often responds through the activation or suppression of various biochemical pathways, which directly affects the synthesis of secondary metabolites.

Secondary metabolites such as alkaloids, flavonoids, tannins, and phenols are key components of plant defence systems. They play multifunctional roles in protecting plants against pathogens, herbivores, and oxidative stress (Al-Khayri *et al.*, 2023). When parasitism occurs, these metabolic processes can either be upregulated as a stress

response or downregulated due to resource depletion. The current study supports the latter for most metabolites, indicating that mistletoe infestation predominantly suppresses secondary metabolite biosynthesis in *Terminalia catappa*.

Alkaloids are nitrogen-containing compounds synthesized primarily through amino acid pathways. Their biosynthesis depends on sufficient nitrogen availability within the host's tissues (Trease and Evans, 2002). The significant reduction in alkaloid levels observed in mistletoe-infested leaves suggests that nitrogen resources are diverted toward the parasite, thereby diminishing nitrogen assimilation in the host. Given that alkaloids often contribute to antimicrobial and anti-inflammatory activities, their reduction may partly explain the decreased medicinal efficacy of mistletoe-infected *T. catappa* leaves.

Flavonoids and phenolic acids are among the most critical antioxidant compounds in plants, neutralizing reactive oxygen species (ROS) generated during metabolic stress (Rice-Evans *et al.*, 1997). In the present study, both metabolites were significantly lower in mistletoe-infested samples, indicating a compromised antioxidant defence system. This could be due to metabolic reallocation as mistletoe siphons off carbohydrates and minerals, the host plant may prioritize primary metabolism over secondary compound production. The decline in phenolic compounds also implies reduced structural defence. Phenolics are precursors for lignin, which strengthens cell walls against pathogen entry. Their depletion may, therefore, render the host more vulnerable to secondary infections. This biochemical vulnerability not only affects plant health but

may also lower the therapeutic value of the leaves when used in herbal formulations (Isikhuemen *et al.*, 2020).

Tannins, a group of polyphenolic compounds, play dual defensive and ecological roles. They deter herbivory by binding to proteins and contribute to antioxidant capacity by neutralizing free radicals (Scalbert, 1991). The present study recorded a significant reduction in tannin levels in mistletoe-infested leaves. Reduced tannin content may be attributed to carbon stress caused by parasitism as mistletoe diverts assimilates, the host experiences limited carbon skeletons for secondary metabolite synthesis.

Unlike the other metabolites, saponins exhibited a slight increase in generally infested leaves (INF-G-L). Saponins are glycosides known for their surface-active and antifungal properties (Francis *et al.*, 2002). Their accumulation is often associated with stress-induced defence signaling, particularly through the jasmonic acid and salicylic acid pathways, which mediate plant responses to biotic attack (Tholl, 2015). The mild elevation in saponin levels observed here may reflect an adaptive response of *T. catappa* to parasitic stress, as the plant attempts to fortify its chemical defences against further infestation. Contrary to the findings of Erhabor *et al.* (2022) who reported higher amount of saponin present in the non-infested species or the low amount of alkaloid in the non-infested species likely responsible for the resistance to mistletoe growth. Therefore, while parasitism suppresses several biosynthetic routes, it can also selectively activate others, leading to non-uniform metabolic adjustments in the host.

Anthraquinones were not detected in any of the samples analysed. This finding aligns with previous studies indicating that *Terminalia catappa* does not synthesize anthraquinones as part of its metabolic repertoire (Anand *et al.*, 2015). The metabolic specialization of *T. catappa* favors the production of ellagitannins, flavonoids, and triterpenoids rather than polyketide-derived compounds like anthraquinones. Thus, their absence in both infested and non-infested leaves reflects species-specific metabolic characteristics rather than parasitic suppression.

5.0 CONCLUSION

This study comparatively evaluated the phytochemical composition of mistletoe-infested and non-infested *Terminalia catappa* leaves to determine the effect of parasitism on secondary metabolite production. The findings revealed that mistletoe infestation significantly reduced the concentrations of alkaloids, flavonoids, phenols, and tannins, while slightly increasing saponins. These results indicate that parasitic stress disrupts the biosynthesis of key phytochemicals in *T. catappa*, likely through nutrient competition and altered metabolic activity. Therefore, the study concludes that mistletoe infestation negatively influences the phytochemical profile and consequently, the medicinal quality of *T. catappa* leaves. Healthy leaves contain higher concentrations of bioactive compounds and are thus more suitable for medicinal and pharmacological use. The results highlight the need for regular monitoring and control of mistletoe infestation to preserve the therapeutic integrity and ecological health of *T. catappa* and related medicinal tree species.

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