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Comparative Phytochemical Profiling of Terminalia catappa, Azadirachta indica and Stachytarpheta Angustifolia Bridging Traditional use and Scientific Validation

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ABSTRACT
This study investigates the qualitative and quantitative phytochemical composition of *Terminalia catappa*, *Azadirachta indica*and *Stachytarpheta angustifolia*, three medicinal plants widely used in traditional medicine. Leaf extracts were obtained using methanol and dichloromethane (DCM) extractions and screened for secondary metabolites. Qualitative analysis confirmed the presence of flavonoids, tannins, alkaloids, saponins, phenolics, and glycosides in varying proportions across all samples. Quantitative analysis revealed that *T. catappa* had the highest concentrations of flavonoids (73.3 ± 2.0 mg/g) and alkaloids (68.5 ± 1.6 mg/g), while *A. indica* exhibited the greatest levels of tannins (67.4 ± 1.8 mg/g) and phenolics (78.3 ± 2.2 mg/g). *S. angustifolia* presented moderate levels of all phytochemicals, with notable flavonoid (62.4 ± 1.7 mg/g) and alkaloid (50.7 ± 1.3 mg/g) content. These findings support the therapeutic potential of the studied species and emphasize their relevance as sources of pharmacologically active compounds.

Keywords:
Terminalia catappa,
Azadirachta indica,
Stachytarpheta Angustifolia,

INTRODUCTION

The utilization of medicinal plants represents one of the most enduring and widespread forms of healthcare, with an estimated 80% of the global population relying on plant-derived therapies for primary health needs (WHO, 2023). This reliance is deeply rooted in history, spanning over 60,000 years, and is experiencing a modern renaissance driven by several critical factors. These include the structural complexity and multi-target therapeutic potential of plant secondary metabolites, their perceived safety profile compared to synthetic drugs, and the escalating global crisis of antimicrobial resistance (AMR), wherein approximately 70% of pathogenic bacteria now demonstrate resistance to at least one antibiotic (Sharma *et al.*, 2019). This urgent need for novel bioactive scaffolds has refocused scientific attention on natural products, with nearly 40% of modern, FDA-approved pharmaceuticals, such as aspirin and paclitaxel, originating from plant precursors (Atanasov *et al.*, 2021).

Ethnobotanical knowledge serves as an invaluable compass in this discovery process, guiding researchers toward species with a long history of human use and validated bioactivity (Heinrich & Jaeger, 2015).

Among the vast array of botanicals used in traditional medicine, *Terminalia catappa L.* (Indian almond), *Azadirachta indica A.* Juss. (neem), and *Stachytarpheta angustifolia* (Mill.) Vahl (narrow-leaf vervain) are particularly noteworthy for their extensive ethnopharmacological applications. *T. catappa* is renowned for its efficacy in managing oxidative stress and diabetes, properties largely attributed to its high concentration of flavonoids and hydrolyzable tannins (Akinmoladun *et al.*, 2020). *A. indica*, often dubbed "the village pharmacy," exhibits potent broad-spectrum antimicrobial and anti-inflammatory activities due to its unique limonoids, such as azadirachtin, and diverse phenolic compounds (Alzohairy, 2016). In contrast, *S. angustifolia* remains relatively understudied but shows significant promise in the treatment of respiratory infections and wound healing, primarily due to its rich content of iridoid glycosides and alkaloids (Olorunnisola *et al.*, 2016).

Despite the well-documented traditional uses of these species, their translation into evidence-based medicine is impeded by significant research gaps.

A primary challenge is the lack of standardized methodologies for phytochemical extraction and analysis,

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leading to considerable variability in reported metabolite concentrations and hindering cross-study comparisons (Ande*et al.*, 2022). Furthermore, quantitative data for key pharmacologically active biomarkers are often insufficient, with established thresholds for efficacy remaining undefined for many compounds (Kumar *et al.*, 2020). Finally, the dynamic nature of phytochemical expression, which is profoundly influenced by environmental factors such as soil composition, seasonality, and plant ontogeny, is frequently overlooked, creating a critical blind spot in chemo-ecological understanding (Olorunnisola *et al.*, 2023).
Therefore, this study addressed these deficiencies by conducting a comprehensive and comparative phytochemical profiling of *T. catappa, A. indica, and S. angustifolia* leaf extracts. Through integrated qualitative and quantitative analyses, we seek to standardize the metabolite composition of these species, thereby validating their traditional uses and establishing a foundational chemical database to support their future development into standardized herbal formulations or sources of novel therapeutic agents.

MATERIALS AND METHODS

Plant Collection and Identification

The leaves of *Terminalia catappa*, *Azadirachta indica,* and *Stachytarpheta angustifolia* were collected from Dutsin-Ma, Katsina State, Nigeria, in May 2025. The plants were identified and authenticated at the Department of Biological Sciences, Federal University Dutsin-Ma, Katsina State. Each species was assigned a voucher specimen code for reference: *Terminalia catappa* (BUKHAN 0389), *Azadirachta indica* (BUK 0211), and *Stachytarpheta angustifolia* (Biological Sciences Gombe State University 102).

Sample Preparation

The collected leaves were air-dried at room temperature for about a week and then ground into a coarse powder using a mortar and pestle. A total of 97.5 grams of powder from each plant was prepared for the extraction process.

Extraction of Plant Materials

Each plant sample was extracted sequentially with dichloromethane (DCM) and methanol. First, 950 mL of DCM was used to extract the plant material for 72 hours, followed by a 72-hour extraction with 500 mL of methanol. The samples were swirled intermittently to enhance extraction. After the extraction process, the solvents were evaporated under reduced pressure at 40°C using a rotary evaporator. The dried extracts were stored in a desiccator at room temperature for further analysis.

Qualitative Phytochemical Analysis

Standard methods from Azwanida(2015) were employed:

- Alkaloids (Mayer's Test): A few drops of Mayer's reagent were added to the extract. The presence of a green precipitate indicated alkaloids.
- Saponins (Foam Test): Distilled water (20 mL) was added to the extract and shaken for 10 minutes. Foam formation confirmed saponins.
- Flavonoids (Acid Test): Diluted sulfuric acid was added to the extract. An orange coloration indicated flavonoids.
- Tannins (Lead Acetate Test): A few drops of 1% lead acetate were added to the extract and shaken. A yellowish precipitate signaled tannins.

- Coumarins: Sodium hydroxide (10%) was added to the extract. A yellow color indicated coumarins.
- Total Phenols: Ferric chloride (3%) was added to the extract. A deep blue color confirmed the presence of phenols.
- Glycosides (Libermann's Test): Chloroform and acetic anhydride were added to the extract. The development of a violet to reddish-brown ring indicated glycosides.

Quantitative Phytochemical Analysis

Methods from Sharma*et al.*, (2019) were used for quantifying the following compounds:

- Phenols: The plant sample was boiled with diethyl ether, followed by addition of distilled water, ammonium hydroxide, and butanol. The reaction mixture was measured at 505 nm using a spectrophotometer.
- Alkaloids: The sample (5 g) was treated with 10% acetic acid in ethanol and allowed to stand for 4 hours. After filtration and partial evaporation, ammonium hydroxide was added to precipitate alkaloids, which were then filtered, washed, dried, and weighed.
- Tannins: The sample (0.5 g) was stirred in distilled water for 1 hour, filtered, and reacted with iron (III) chloride and potassium ferrocyanide. Absorbance was read at 395 nm.
- Flavonoids: The sample (10 g) was extracted with 80% methanol, filtered, evaporated to dryness, and weighed until a constant mass was achieved.

High-Performance Liquid Chromatography (HPLC) Analysis

HPLC was employed for detailed compound identification and quantification. The HPLC analysis was performed using a C18 reverse-phase column with a mobile phase of acetonitrile and water (0.1% formic acid). The flow rate was maintained at 1.0 mL/min, with detection at 254 nm for flavonoids and 280 nm for alkaloids and terpenoids.

RESULTS AND DISCUSSION

Results on Qualitative Phytochemical Screening
Table 1: Qualitative Phytochemical Screening

Phytochemicals	*T. catappa*	*A. indica*	*S. angustifolia*
Tannins	+++	++++	++
Saponins	++	++	++
Flavonoids	++++	++	+++
Alkaloids	+++	++	++
Glycosides	++	++	+++
Phenolics	+++	++++	++

Key: Legend: + = trace, ++ = moderate, +++ = abundant, ++++ = very abundant

Table 2: Quantitative Phytochemical Content (mg/g dry weight)

Compound	*T. catappa*	*A. indica*	*S. angustifolia*
Tannins	52.1 ± 1.2	67.4 ± 1.8	34.6 ± 0.9
Saponins	24.7 ± 0.8	23.9 ± 0.5	22.1 ± 0.6
Flavonoids	73.3 ± 2.0	45.8 ± 1.5	62.4 ± 1.7
Alkaloids	68.5 ± 1.6	54.2 ± 1.4	50.7 ± 1.3
Phenolics	59.4±1.1	78.3±2.2	47.6±1.5

The comprehensive phytochemical profiling conducted in this study as shown in table 1 and 2, provides robust chemical validation for the traditional medicinal uses of *Terminalia catappa*, *Azadirachta indica, and Stachytarpheta angustifoli*a. The quantitative and qualitative data align closely with the documented ethnopharmacological applications and previously reported biological activities of these species, while also highlighting critical factors influencing their phytochemical composition.

Pharmacological Correlates of Phytochemical Composition

The superior flavonoid (73.3 ± 2.0 mg/g) and alkaloid (68.5 ± 1.6 mg/g) content in *T. catappa* leaf extracts offers a clear biochemical basis for its renowned antioxidant and antimicrobial properties. The high flavonoid concentration directly correlates with potent free radical scavenging activity, such as the low IC~50~ value (12.4 μg/mL) against DPPH radicals reported elsewhere (Akinmoladun *et al.,* 2020). This efficacy is mechanistically linked to the ability of flavonoids to donate hydrogen atoms and chelate pro-oxidant metal ions, thereby mitigating oxidative stress associated with diabetes and neurodegenerative pathologies. Concurrently, the significant alkaloid content likely contributes to the observed broad-spectrum antimicrobial efficacy against pathogens like Escherichia coli (Mwangi *et al.*, 2024), potentially through mechanisms involving microbial cell membrane disruption and inhibition of nucleic acid synthesis.

In contrast, *A. indica* exhibited dominance in phenolic (78.3 ± 2.2 mg/g) and tannin (67.4 ± 1.8 mg/g) content, which substantiates its extensive use in dermatological and wound care formulations, often earning it the epithet "the village pharmacy." Phenolic compounds are known to enhance wound healing by promoting collagen cross-linking and stimulating angiogenesis (Alzohairy, 2016), while tannins impart astringent effects that reduce exudation and inflammation in chronic wounds. The high yield of phenolics is consistent with extraction efficiency in solvents of intermediate polarity. However, the well-documented batch-to-batch variability of key biomarkers like azadirachtin- reportedly up to 40% (Airaodion*et al.,* 2019) -underscores a significant challenge in natural product standardization and necessitates the development of optimized, stability-indicating extraction protocols.

S. angustifolia presented a more balanced phytochemical profile, with notably moderate to high levels of flavonoids and alkaloids. Its significant glycoside content, as indicated qualitatively, is of particular pharmacological interest. These compounds, specifically phenylethanoid glycosides like verbascoside (identified via TLC at R~f~ 0.28), are strongly associated with anti-inflammatory activity via inhibition of the NF-KB pathway and subsequent reduction of pro-inflammatory cytokines such as TNF-a (Olorunnisola *et al.,* 2016). The COX-2 inhibitory activity (IC~50~ 18 μM) of verbascoside (Tran *et al.,* 2023) provides a mechanistic rationale for the traditional use of *S. angustifolia* in treating respiratory infections and inflammatory conditions.Furthermore, the observed phytochemical variability, particularly the lower tannin content in *S. angustifolia* compared to *A. indica*, may be attributed to ecotypic and edaphic factors. Soil nutrient composition, especially nitrogen deficiency, has been demonstrated to suppress phenolic biosynthesis by 30-50% (Kumar *et al.,* 2020). This highlights the profound influence of chemo-ecological factors on phytochemical yield and emphasizes the need for future studies to integrate soil nutrient mapping and control for seasonal variation-for instance, harvesting *S. angustifolia* during flowering, when verbascoside levels may increase by 300% (Olorunnisola *et al.,* 2023).

The choice of extraction solvent proved critical for the recovery of specific metabolite classes. Methanol, due to its high polarity (dielectric constantε = 33), was highly effective for solubilizing polar flavonoids, whereas its use may be suboptimal for less polar terpenoids and phenolics, which were also detected in DCM extracts. While traditional maceration was employed here for its accessibility, advanced techniques such as Ultrasound-

Assisted Extraction (UAE) could potentially enhance extraction yields by 20-40% through cavitation-induced cell wall rupture (Nwali *et al.*, 2018), representing a significant improvement for future industrial applications.

Thin-Layer Chromatography (TLC) Analysis

TLC analysis under UV light (254 nm and 365 nm) revealed distinctive phytochemical bands.

Table 3: LC Rf Analysis and Inference

Plant Extract	Spot	Rf	Compound Class	Interpretation
T. catappa DCM	T1	0.32	Terpenoids	Indicates presence of lipophilic bioactives
	T2	0.48	Flavonoids	Confirmed polar constituents in non-polar extract
A.indica Methanol	A1	0.24	Phenolic acid	Strong polar component presence
	A2	0.46	Azadirachtin derivatives	Consistent with known neem phytochemicals
S. angustifolia DCM	S1	0.28	Iridoid glycosides	Semi-polar compounds present in DCM extract
	S2	0.46	Alkaloids	Common antimicrobial secondary metabolites

From table 3, the Thin-Layer Chromatography (TLC) profiling analysis provided valuable preliminary validation of the phytochemical classes present. The distinct R~f~ values confirmed efficient separation and were consistent with known standards: the low R~f~ value (0.24) of phenolic acids in *A. indica* reflects their high polarity, while the terpenoid spot (R~f~ 0.32) in *T. catappa* aligns with the migration pattern of ursolic acid (Lin et al., 2021). The alkaloid spot (R~f~ 0.46) in *S. angustifolia* is consistent with stachytarphetine-type compounds, further supporting the antimicrobial potential of this species.The DCM extract of *Terminalia catappa* leaves contains significant phytochemical bands indicative of terpenoids and flavonoids, which contribute to its pharmacological properties. These findings validate the plant's traditional medicinal uses and provide a scientific basis for its incorporation into modern pharmacological practices. The methanol extract of *Azadirachta indica* leaves contains significant phytochemical bands indicating the presence of phenolic acids and azadirachtin derivatives, which contribute to its medicinal properties. These findings confirm the plant's traditional uses and provide a scientific basis for its incorporation into modern pharmacological treatments (Benisheikh 2019). The DCM extract of *Stachytarpheta angustifolia* contains significant bands indicative of iridoid glycosides and alkaloids, which contribute to its pharmacological properties. These findings validate the traditional medicinal uses of the plant and provide a scientific foundation for its use in modern pharmacological treatments.

CONCLUSION

Phytochemical profiles of *Terminalia catappa*, *Azadirachta indica*, and *Stachytarpheta angustifolia* highlights their significant pharmacological potential, aligning with their widespread use in traditional medicine. The study not only supports their therapeutic uses but also lays a scientific foundation for their inclusion in modern pharmacological treatments. *Terminalia catappa* displayed a variety of bioactive compounds, including triterpenoids, flavonoids, and tannins, which contribute to its antioxidant, anti-inflammatory, and antimicrobial properties. Similarly, *Azadirachta indica* was found to contain a range of phytochemicals such as alkaloids, flavonoids, and saponins, which are linked to its antidiabetic, anti-inflammatory, and antimicrobial effects. *Stachytarpheta angustifolia* was found to contain alkaloids, flavonoids, tannins, saponins, and terpenoids, which are believed to be responsible for its analgesic, anti-inflammatory, and antimicrobial properties. These results corroborate its traditional uses and provide scientific backing for its therapeutic benefits. Further research, particularly clinical trials, is needed to confirm the safety and effectiveness of these plant extracts and to explore their potential applications in drug development and modern therapies.

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