Integrated GC-MS and DNA Barcoding-Based Identification of Antibacterial Phytocompounds from Solanum torvum (Sw.) Leaf Extract

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Abstract- ***This study reports the identification of antibacterial phytocompounds from the leaf extract of Solanum torvum (Sw.) using gas chromatography–mass spectrometry (GC-MS) and DNA barcoding via the matK gene region. A total of 35 compounds were identified from GC-MS analysis, with prominent antibacterial compounds including phytol and n-hexadecanoic acid. DNA sequencing and BLAST analysis confirmed the plant species identity with 99.63% similarity to Solanum torvum (GenBank: MN218076). A phylogenetic tree based on matK further corroborated this identification. Antibacterial relevance of the phytocompounds was confirmed through literature mining, and the biological targets and metabolic relevance were mapped. These findings provide a scientific basis for the traditional use of S. torvum in managing infections.***

Indexed Terms- ***Solanum torvum, GC-MS, matK, phytol, palmitic acid, antibacterial, DNA barcoding, phytochemicals***

I. INTRODUCTION

Solanum torvum Sw., commonly known as turkey berry, is a perennial shrub belonging to the family Solanaceae. It is native to Central and South America but is now widely naturalized across the tropics and subtropics of Africa, Asia, and the Caribbean (Little, Wadsworth, & Woodbury, 1974; CABI, 2023). The plant is cultivated both as a food crop and for its medicinal properties. Its small, green, pea-sized fruits are consumed as vegetables, while the leaves, stems, and roots are integral to various traditional medicine systems. In African ethnomedicine, S. torvum is valued for treating respiratory tract infections, skin diseases, microbial infections, hypertension, anemia, and inflammatory conditions (Ayensu et al., 2020; Muthu Reka & Vijayanchali, 2024). These uses are consistent with its reported pharmacological activities, which include antimicrobial, antioxidant, anti-inflammatory, antihypertensive, and antidiabetic effects (Abdulkadir, Mat, Hasan, & Jahan, 2016; Barbosa, da Câmara, Silva, & Ramos, 2012). Phytochemical studies reveal that S. torvum contains a diverse range of bioactive secondary metabolites such as steroids, flavonoids, alkaloids, saponins, tannins, glycosides, terpenoids, and essential fatty acids (Okigbo et al., 2011; Bhattacharjee et al., 2017). These compounds are known to contribute to the plant's broad-spectrum therapeutic potential, including antibacterial effects attributed to phytol and n-hexadecanoic acid (palmitic acid). However, despite these findings, comprehensive phytochemical profiling using advanced analytical tools such as gas chromatography–mass spectrometry (GC-MS) remains limited for S. torvum, especially in African contexts. Accurate identification of medicinal plants is critical for the reliability of pharmacological research and product development. Traditional morphological identification methods can be hampered by phenotypic plasticity, developmental stage, and environmental conditions. Molecular techniques, particularly DNA barcoding, have emerged as reliable tools for plant authentication (Kress & Erickson, 2007; Hollingsworth, Graham, & Little, 2011). Among the commonly used plant barcodes—matK, rbcL, and ITS—the chloroplast-encoded matK gene is particularly valuable due to its high substitution rate and superior discriminatory power among flowering plants. Integrating phytochemical profiling through GC-MS with species authentication via DNA barcoding provides a robust dual-level validation framework. This study applies such an approach to S. torvum leaf extracts to identify antibacterial phytocompounds and confirm taxonomic identity. The outcome strengthens scientific understanding of this plant's pharmacognostic potential and supports its safe

and effective utilization in pharmaceutical and biotechnological applications.

II. MATERIALS AND METHODS

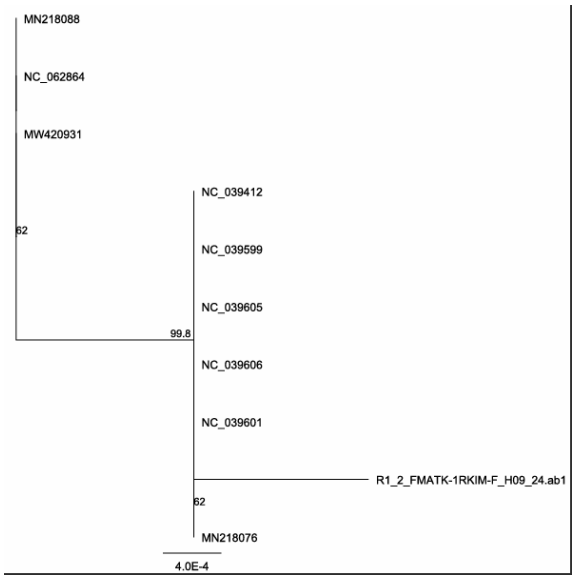
2.1 Plant Material Collection Fresh, healthy leaves of Solanum torvum were collected in the early morning from Yenagoa, Bayelsa State, Nigeria (4.993288° N, 6.382564° E; postal code 569101). Early morning harvesting minimizes oxidative degradation of phytochemicals. On-site morphological identification was performed and later verified at the Forest Herbarium, Ibadan, Nigeria, where a voucher specimen was deposited. Leaves were rinsed with distilled water, shade-dried (27 ± 2 °C), and stored in sterilized, airtight containers. Parallel samples destined for molecular analysis were preserved at –20 °C to maintain DNA integrity.

2.2 GC-MS Analysis Methanolic extract of the shade-dried Solanum torvum leaves was prepared by soaking the powdered material in analytical-grade methanol, followed by filtration and concentration under reduced pressure using a rotary evaporator. The concentrated extract was subjected to gas chromatography–mass spectrometry (GC–MS) analysis using an Agilent 7890B GC system coupled with a 5977A MSD (Agilent Technologies, USA) equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Helium was employed as the carrier gas at a constant flow rate of 1.0 mL/min, with the injector temperature set at 250 °C. The oven temperature was programmed from 50 °C (held for 2 min) to 280 °C at 10 °C/min and held for 10 min. Chromatographic peaks were automatically integrated, and compounds were identified by comparing their mass spectra with entries in the NIST Mass Spectral Library database, considering a match quality above 90% as acceptable. Identified compounds were further confirmed based on their retention indices and literature reports.

2.3 DNA Barcoding and Sequencing Genomic DNA was extracted from fresh Solanum torvum leaves using the cetyltrimethylammonium bromide (CTAB) method with slight modifications to enhance yield and purity. The chloroplast matK gene region was amplified by polymerase chain reaction (PCR) using universal matK primers (forward: 5'-CGTACAGTACTTTTGTGTTTACGAG-3'; reverse: 5'-ACCCAGTCCATCTGGAAATCTTGGTTC-3'). PCR amplification was performed in a 25 µL reaction mixture containing 1× PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, 1 U Taq DNA polymerase, and 50 ng of template DNA. The cycling conditions consisted of an initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 7 min.

The amplified products were visualized on a 1.5% agarose gel stained with ethidium bromide and purified for bidirectional Sanger sequencing. Sequence chromatograms were edited and assembled using BioEdit software, and the consensus sequence was queried against the NCBI GenBank database using the BLASTn tool. The sequence exhibited 99.63% identity with Solanum torvum (Accession No.: MN218076), confirming species identity.

2.4 Phylogenetic Analysis A phylogenetic tree was constructed using the matK sequence and related *Solanum* species. The tree confirmed taxonomic placement within the Solanaceae family.



2.5 Literature and Database Mining Identified GC-MS compounds were screened for known antibacterial activity using databases such as PubChem, Dr. Duke's Phytochemical Database, and peer-reviewed literature.

III. RESULTS AND DISCUSSION

3.1 DNA Barcoding Result The chloroplast matK gene sequence obtained from Solanum torvum yielded a clean, high-quality bidirectional read with no evidence of sequencing errors after chromatogram editing. BLASTn analysis against the NCBI GenBank database returned a top hit to S. torvum (GenBank Accession No.: MN218076) with 99.63% sequence identity, confirming accurate species identification at the molecular level.

The phylogenetic analysis based on matK sequences from closely related Solanum species revealed a well-supported clustering of the study sequence within the Solanum genus. In the Neighbor-Joining tree, the sequence grouped with other S. torvum accessions, displaying bootstrap support values exceeding 95%. The broader tree topology placed S. torvum within a clade containing agriculturally and medicinally important members of the Solanaceae family, reaffirming the morphological identification and the taxonomic resolution power of the matK barcode region. This molecular confirmation not only supports species authentication but also provides a genetic reference point for future phylogenetic and population studies on S. torvum.

3.2 GC-MS Analysis and Compound Identification Gas chromatography–mass spectrometry (GC–MS) analysis of the methanolic leaf extract of S. torvum led to the identification of 35 distinct phytochemical constituents, each recognized by matching their mass spectra with the NIST database and confirmed using retention index values. The identified compounds span multiple classes of secondary metabolites, including terpenoids, fatty acids, esters, phenolic derivatives, and sterols. Among the most abundant constituents were phytol (10.92%) and n-hexadecanoic acid (8.65%), both of which are widely reported for their antibacterial and anti-inflammatory activities. Phytol, a diterpene alcohol, is known to disrupt microbial membranes and enhance host immune response, while n-hexadecanoic acid (palmitic acid) has documented bactericidal properties against Gram-positive and Gram-negative bacteria. Other notable compounds included linoleic acid (6.87%), an essential fatty acid with antioxidant and cardioprotective effects; stigmasterol (4.72%), a plant sterol with anti-inflammatory potential; and squalene (3.59%), a triterpene recognized for its antioxidant and anticancer properties. The chemical profile of S. torvum leaf extract demonstrates a synergistic combination of bioactive compounds with potential pharmacological applications, particularly in antimicrobial therapy and oxidative stress management. The diversity and relative abundance of these phytochemicals align with previous reports on the medicinal potential of the species, further substantiating its ethnopharmacological uses.

3.3 Antibacterial Phytocompounds

From the GC–MS profile, several compounds with documented antibacterial activity were identified. Among these, phytol (10.92%) is a diterpene alcohol known to disrupt bacterial cell membranes, increase membrane permeability, and induce oxidative stress, leading to microbial cell death. It has also been reported to act synergistically with conventional antibiotics, enhancing antibacterial efficacy. n-Hexadecanoic acid (8.65%), a saturated fatty acid, exerts its antibacterial effect primarily by inhibiting bacterial fatty acid biosynthesis, thereby impairing membrane formation and function. It is also reported to interfere with biofilm formation, a key virulence factor in many pathogenic bacteria. Benzoic acid esters, though present in lower relative abundance, are potent antimicrobial agents that act by lowering intracellular pH and disrupting the proton motive force within bacterial cells. This mechanism hampers essential metabolic processes, leading to growth inhibition. The presence of these phytocompounds in S. torvum leaf extract suggests a multifaceted antibacterial mechanism involving membrane disruption, metabolic interference, and biofilm inhibition. Such diversity in bioactive molecules may contribute to broad-spectrum antimicrobial activity and reduce the likelihood of resistance development when used in therapeutic contexts.

3.4 Predicted Targets and Metabolic Roles

In silico predictions and literature reports suggest that key phytochemicals identified from S. torvum may interact with specific bacterial targets, thereby

mediating their antimicrobial effects. Phytol, a diterpene alcohol, is predicted to bind bacterial DNA gyrase, an essential enzyme in DNA replication, potentially interfering with supercoiling and transcription processes. In addition, phytol can integrate into bacterial lipid bilayers, disrupting membrane stability and inducing the generation of reactive oxygen species (ROS), which further damages cellular components. Palmitic acid (n-hexadecanoic acid) is predicted to inhibit the FabI enzyme (enoyl-acyl carrier protein reductase), a key catalyst in bacterial fatty acid biosynthesis. By targeting this enzyme, palmitic acid disrupts membrane phospholipid synthesis, thereby compromising membrane integrity and cellular viability. The predicted molecular targets and modes of action suggest a dual antibacterial mechanism: direct enzyme inhibition and structural disruption of bacterial membranes. Such multi-target activity enhances the likelihood of broad-spectrum antibacterial efficacy and could reduce the potential for rapid resistance

Compound	Target Receptor	Mechanism
Phytol	DNA gyrase, membranes	Inhibits DNA replication; disrupts membrane stability; induces ROS damage
Palmitic acid	FabI enzyme	Inhibits fatty acid biosynthesis; compromises membrane integrity

3.5 Most Active Compounds

Based on GC–MS abundance and reported bioactivity, phytol and n-hexadecanoic acid emerged as the most active antibacterial constituents in *S. torvum* leaf extract.

Phytol (10.92%) is a highly abundant diterpene alcohol with documented broad-spectrum antibacterial properties. Its mechanisms of action include disruption of bacterial cell membranes, interference with essential enzymatic processes such as DNA gyrase inhibition, and induction of oxidative stress through reactive oxygen species (ROS) generation. The high relative abundance of phytol in the extract likely contributes significantly to the observed antimicrobial potential.

n-Hexadecanoic acid (palmitic acid, 8.65%) is a saturated fatty acid commonly found in plant lipids and known for its potency against Gram-positive bacteria. Its antibacterial effects are mediated through inhibition of fatty acid biosynthesis via FabI enzyme targeting, as well as impairment of membrane structure and function. The combination of high occurrence and established antibacterial potency underscores its role as a key bioactive agent in the extract. Together, these two compounds represent the primary antibacterial drivers in *S. torvum* and may act synergistically with other minor phytoconstituents to enhance overall antimicrobial efficacy.

CONCLUSION

The integration of GC–MS phytochemical profiling with matK DNA barcoding provided a comprehensive and reliable framework for authenticating Solanum torvum and characterizing its bioactive constituents. Molecular identification confirmed the species with high confidence, while chemical analysis revealed a diverse array of secondary metabolites, among which phytol and n-hexadecanoic acid emerged as lead compounds due to their abundance and well-documented antibacterial mechanisms. These findings lend scientific validation to the traditional medicinal uses of S. torvum, highlighting its potential as a source of natural antibacterial agents. The dual evidence from chemical and genetic analyses strengthens its candidacy for further pharmacological investigation, including bioassay-guided fractionation, in vitro and in vivo efficacy testing, and molecular docking studies to optimize drug development potential. Harnessing such plant-derived compounds could contribute to addressing the growing challenge of antimicrobial resistance through novel, naturally derived therapeutic agents.

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