

Gas chromatographic–mass spectroscopic (Gc-MS) quantification of volatile constituents of ethanol extract of *Andrographis paniculata* collected from Nekede, Imo State, Nigeria

Iwalola M. K.¹, Ihejieta H. A.², Nkwocha G. A.^{3*}, Ekenyem P. C.¹, Anukam, K.U.⁴ and Edih, M.C.⁴

¹Department of Animal Health and Production Technology, Federal Polytechnic, Nekede, Nigeria.

²Department of Microbiology and Biochemistry, Federal Polytechnic, Nekede, Nigeria.

³Faculty of Agriculture, Alex Ekwueme Federal University, Ndufu-Alike, Ikwo, Abakaliki, Ebony state, Nigeria.

⁴Department of Animal Production and Health Technology, Imo State Polytechnic, Omuma, P.M.B.1472, Owerri, Nigeria.

*Corresponding author. Email: geffmacnkwo@gmail.com; Tel: +2348035078259.

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ABSTRACT: *Andrographis paniculata* is a globally recognised medicinal plant widely used for its anti-inflammatory, antimicrobial, and metabolic regulatory properties. While existing pharmacological literature has largely focused on its polar diterpenoid lactones, such as andrographolide, there remains a critical research gap concerning the plant's volatile and semi-volatile constituents. This study addresses this gap by providing a comprehensive GC–MS-based phytochemical profile of an ethanolic extract of *A. paniculata* collected from Nekede, Imo State, Nigeria. Environmental factors such as soil nutrient availability and local weather patterns can alter the plant's secondary metabolic pathways, resulting in distinct regional chemotypes. GC–MS analysis detected 86 bioactive components, resolved into 32 chromatographic peaks. Aromandendrene (6.12%) was identified as the dominant sesquiterpene marker for this Nigerian variety. This finding differs from studies of Asian origins, which typically reported 60–65 bioactive components. Other major bioactive markers included linalool (2.45%), γ -elemene (2.23%), and γ -terpinene (1.34%). The identification of these volatile terpenoids provides essential baseline fingerprint data for the quality control and standardisation of *A. paniculata* within the Nigerian ecosystem. Furthermore, these constituents exhibit potent antioxidant, antimicrobial, and immunomodulatory activities, highlighting the plant's potential as a phytochemical alternative to synthetic antibiotics in livestock and poultry production. This study concludes that the therapeutic value of *A. paniculata* arises from a multi-component synergy between its well-known lactones and these newly characterised volatile markers.

Keywords: *Andrographis paniculata*, Medicinal plants, phytochemical profiling, terpenoids, volatile compounds.

INTRODUCTION

Andrographis paniculata, belonging to the family Acanthaceae, is a well-known medicinal plant widely distributed in Asia and parts of Africa. It is commonly used in traditional medicine systems for the treatment of fever, respiratory infections, gastrointestinal disorders, inflammation, diabetes, and cardiovascular diseases (Chao and Lin, 2010; Kalaivani *et al.*, 2012). The therapeutic properties of *A. paniculata* have largely been

attributed to its diterpenoid lactones, particularly andrographolide and its derivatives, including neoandrographolide and 14-deoxy-11, 12-didehydroandrographolide (Akowuah *et al.*, 2006). Advanced chromatographic techniques such as HPLC, UPLC, LC–MS, and RRLC–TOF/MS have been extensively applied for the quantitative determination of these polar diterpenoids (Villedieu-Percheron *et al.*, 2019;

Dwivedi *et al.*, 2021). However, volatile and semi-volatile phytochemicals, which may contribute to antioxidant, antimicrobial, insecticidal, and synergistic pharmacological effects, are less frequently explored. Gas chromatography–mass spectrometry (GC–MS) remains one of the most reliable analytical tools for profiling volatile and low-polarity compounds in medicinal plants (Chrivastava *et al.*, 2025). Previous GC–MS studies on *A. paniculata* have reported the presence of terpenoids, fatty acids, hydrocarbons, and sterols, suggesting a chemically diverse phytochemical profile beyond andrographolide-based constituents (Kalaivani *et al.*, 2012; Sithara and Nair, 2017). The present study therefore was aimed to conduct a GC–MS-based qualitative and relative quantitative analysis of volatile constituents in an ethanolic extract of *Andrographis paniculata* collected from the Nekede region of Imo State, Nigeria, to compare with the existing LC-based phytochemical data and contribute to plant standardisation efforts.

The bioactive volatile constituents identified under study in *Andrographis paniculata* have strong relevance in veterinary medicine, livestock production, and poultry health, particularly as alternatives or complements to synthetic drugs (Redlarska *et al.*, 2025). Their activities align with major challenges in animal health, including oxidative stress, inflammation, infectious diseases, immune suppression, parasitism, and poor growth performance (Villedieu-Percheron *et al.*, 2019).

Linalool has been extensively reported for its antimicrobial, anti-inflammatory, antioxidant, and cardio-protective effects, which are directly relatable to its application to animal production systems. In livestock and poultry, oxidative stress and inflammation are major contributors to reduced growth performance, immune suppression, and increased disease susceptibility. Linalool mitigates oxidative stress by scavenging free radicals and reducing lipid peroxidation in tissues, thereby improving organ function and overall animal performance (Peana *et al.*, 2002). In poultry and small ruminants, linalool-containing plant extracts have demonstrated broad-spectrum antimicrobial activity against *Escherichia coli*, *Salmonella spp.*, and *Staphylococcus aureus*, making it valuable for controlling enteric and respiratory infections (Soković *et al.*, 2010). Its vasorelaxant and cardioprotective effects further suggest benefits in stress-prone animals, especially under intensive production systems.

γ -Terpinene is a potent natural antioxidant that plays a protective role against oxidative tissue damage. In animal health, oxidative stress is commonly associated with heat stress, disease challenge, vaccination stress, and intensive feeding regimens. Studies have shown that γ -terpinene enhances endogenous antioxidant enzyme activity, including superoxide dismutase and catalase, thereby protecting liver and muscle tissues in animals (Ruberto and Baratta, 2000). Following the ban in European, American and some African countries on the

use of antimicrobials to promote poultry growth and fight infections, there has been an increase in poultry digestive diseases due to dysbiosis, that is, an imbalance in gut microbiome. Poor gastrointestinal (GI) health may result in nutrient malabsorption and attendant growth depression in affected poultry birds (Bailey, 2011).

α -Copaene and related sesquiterpenes contribute to anti-inflammatory, antioxidant, and insect-repellent activities. These properties are valuable in controlling ectoparasites (ticks, mites, flies) and reducing inflammation associated with parasitic infestations in livestock (da Silva *et al.*, 2020).

γ -Elemene is one of the most pharmacologically significant sesquiterpenes identified in the extract. It is well known for its anticancer, immunomodulatory, anti-inflammatory, and antiparasitic properties (Jiang *et al.*, 2017). In veterinary applications, γ -elemene has shown potential in modulating immune responses, enhancing macrophage activity, and regulating cytokine production (Zhou *et al.*, 2013). These effects are particularly relevant in poultry exposed to coccidial and bacterial infections and ruminants facing chronic inflammatory and parasitic diseases.

This GC-MS study addresses literature gaps by profiling overlooked volatiles in *A. paniculata* from Nekede, Nigeria, establishing a region-specific chemical fingerprint that complements LC data and links constituents to oxidative stress and enteric infections in animal production.

MATERIALS AND METHODS

Plant material and authentication

Fresh aerial parts of *Andrographis paniculata* were collected from the Forestry Unit of Federal Polytechnic, Nekede, Imo State, Nigeria. The plant material was authenticated by a qualified Taxonomist in the Department of Biology, Federal University of Technology Owerri (FUTO), Imo State, Nigeria, with authentication number FHI 109503 and a voucher specimen was deposited in the herbarium for future reference.

Preparation of plant extract

Fresh leaves of *Andrographis paniculata* were collected from the Teaching and Research Farm of the Federal Polytechnic, Nekede, Owerri. The plant material was washed under running tap water, air-dried for five (5) days and subsequently pulverised into a fine powder using an industrial blender.

The resulting powder was stored in an airtight container until further use. For extract preparation, 20 g of the dried powder was added to 200 ml of 98% ethanol. The mixture was thoroughly shaken in a sealed container twice every 24 hours. After 48 hours, the mixture was filtered using

Whatman No 1 filter paper. The filtrate was concentrated using a rotary evaporator at 60° C for 30 minutes. The concentrated extract was collected, measured, and stored in an airtight container until further analysis.

Extraction of phytochemicals

1g of the extract was weighed and transferred to a test tube, and 25 ml of ethanol was added. The test tube was allowed to react on a hotplate at 60°C for 90 minutes. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20 ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the funnel. These extracts were combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate, and the solvent was evaporated. The sample was solubilised in 1000 µL of pyridine, of which 200 µL was transferred to a vial for analysis.

Quantification by GC-MS

The analysis of phytochemicals was performed on a BUCK M910 gas chromatograph equipped with an HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionisation system which utilised high-energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with a flow rate of 1 mL/min. The initial temperature was set at 50°C with an increasing rate of 3 °C/min and a holding time of about 10 minutes. Finally, the temperature was increased to 300°C at 10 °C/min. One microliter of the prepared 1% of the extracts diluted with acetonitrile was injected in a splitless mode. Relative quantity of the chemical compounds present in each of the extracts was expressed as a percentage based on peak area produced in the chromatogram.

Identification of chemical constituents

Bioactive compounds extracted from different extracts were identified based on GC retention time on an HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC–MS systems).

RESULTS

GC–MS chromatographic profile of *Andrographis paniculata* in Nekede environ

The GC–MS total ion chromatogram (TIC) of the ethanolic

Table 1. Qualitative studies on dried *A. paniculata* leaf extract

s/no	Phytochemicals discovered	Availability
1.	Phenol	Present
2.	Tannin	✓
3.	Hydrolysable tannin	✓
4.	Flavonoids	✓
5.	Terpenoids	✓
6.	Saponin	✓
7.	Glycoside	✓
8.	Alkaloids	✓

extract of *Andrographis paniculata* revealed thirty-two (32) well-resolved peaks within a retention time range of approximately 6.5–19.0 minutes, indicating a chemically diverse volatile fraction. The detected compounds were predominantly monoterpenes, sesquiterpenes, and aliphatic hydrocarbons, with terpenoid compounds accounting for the majority of the total peak area.

Identification and relative abundance of bioactive compounds

The bioactive constituents were tentatively identified by comparison of their mass spectra with the NIST14 mass spectral library, and their relative abundance was expressed as percentage peak area. The major and biologically relevant compounds are presented in Table 1, while Figure 1 shows the chromatography of *A. paniculata* aerial part in ethanol extract. Analysis of the chemical classes revealed that:

1. Sesquiterpenes were the dominant group, accounting for the highest cumulative peak area
2. Monoterpenes such as linalool and γ-terpinene contributed significantly to the antioxidant and anti-inflammatory profile
3. Aliphatic hydrocarbons were present in low proportions and are commonly reported as background constituents in GC–MS plant analyses

This distribution aligns with earlier GC–MS studies on *Andrographis paniculata* and supports the role of volatile terpenoids as complementary bioactive agents alongside diterpenoid lactones

DISCUSSION

The GC–MS profiling of *Andrographis paniculata* under study (Tables 2 and 3) revealed several pharmacologically important monoterpenes and sesquiterpenes that are known to contribute to the plant's therapeutic efficacy (Aggarwal *et al.*, 2025). Although diterpenoid lactones such as andrographolide are considered the principal bioactive markers, increasing evidence suggests that

Table 2. Quantitative GC-MS results of dried *A. paniculata* leaf extract from Nekede

S/No	Compounds detected	CAS No.	Retention time	% Area
1	Oxirane, (chloromethyl) Decane	000106-89-8	6.496	0.43
2	Benzene, 1,4-dichloro	000106-46-7	6.848	0.22
3	p-Cymene	000099-87-6	7.202	0.21
4	gamma.-Terpinene	000099-85-4	8.162	1.34
5	Octane, 3,4,5,6-tetramethyl-	062185-21-1	8.251	0.22
6	Decane, 2,6,7-trimethyl	062108-25-2	8.380	0.34
7	Nonane	000111-84-2	8.534	0.51
8	Tetradecane	000629-59-4	8.591	0.19
9	Undecane, 2-methyl	007045-71-8	8.644	0.45
10	Heptadecane, 2,6,10,14-tetramethyl	018344-37-1	8.957	2.12
11	Octane, 4-ethyl	015869-86-0	9.118	0.40
12	Tetradecane	000629-59-4	9.176	0.34
13	Heptadecane, 2,6,10,14-tetramethyl	018344-37-1	9.272	0.78
14	Tridecane	000629-59-4	9.339	0.65
15	Linalool	00078-70-6	9.441	2.45
16	Heptadecane, 2,6,10,14-tetramethyl	018344-37-1	9.691	0.67
17	Oxalic acid, 2-ethylhexyl hexyl ester	1000309-38-9	9.735	0.34
18	Nonane, 3-methyl-	005911-04-6	9.808	0.34
19	Undecane, 5-methyl	001632-70-8	10.027	0.11
20	Octane, 2-methyl- 2,6-Dimethyldecane	003221-61-2	10.101	0.23
21	1-Dodecene	000112-41-4	12.028	0.12
22	Dodecane	000112-40-3	12.262	0.44
23	Tridecane	000629-50-5	15.110	0.54
24	alpha.-Cubebene	017699-14-8	16.471	0.34
25	alfa.-Copaene	1000360-33-0	17.185	1.12
26	2,5-Octadiene	063216-69-3	17.645	2.23
27	Decane, 2,3,5-trimethyl-	062238-11-3	17.834	0.13
28	1H-Cycloprop[e]azulene	000489-40-7	18.083	0.46
29	1,3,6,10-Dodecatetraene	026560-14-5	18.251	0.54
30	Aromandendrene	000489-39-4	18.364	6.12
31	Santolina triene 1,3-Cyclopentadiene	002153-66-4	18.737	0.45
32	(1R,3aS,8aS)-7-Isopropyl-1,4-dimethyl	036577-33-0	18.980	0.28
33	Humulene	006753-98-6	19.251	1.34
34	(1R,3aS,8aS)-7-Isopropyl-1,4-dimethyl	036577-33-0	18.980	0.28
35	Humulene, Cyclohexene, 4-[(1E)-1,5-dimethyl	006753-98-6	19.251	1.34
36	Bicyclo[7.2.0]undec-4-ene	000118-65-0	19.478	0.23
37	Naphthalene, 1,2,4a,5,6,8a-	030021-74-0	19.872	0.46
38	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene	013744-15-5	19.982	3.34
39	Naphthalene, 1,2,3,4,4a,5,6,8a	000473-13-2	20.114	2.23
40	Bicyclo[3.1.1]hept-2-ene	017699-05-7	20.349	2.54
41	alpha.-Murolene	010208-80-7	20.481	0.37
42	8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene	1000193-62-7	20.588	0.25
43	(E)-.beta.-Farnesene	018794-84-8	20.718	10.89
44	2,4-Di-tert-butylphenol	000096-76-4	20.982	1.45
45	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene	020307-83-9	21.089	7.59
46	E)-1-Methyl-4-(6-methylhept-5-en-2-ylidene)	053585-13-0	21.260	0.45
47	Cyclohexene, 4-[(1E)-1,5-dimethyl-1,4-hexadien-1-yl]	025532-79-0	21.531	0.53
48	Cyclohexanemethanol	000639-99-6	21.748	0.30
49	Alloaromadendrene Azulene	025246-27-9	21.881	1.23
50	1,6,10-Dodecatrien-3-ol	007212-44-4	22.078	2.59
51	1H-Cycloprop[e]azulen-7-ol	006750-60-3	22.450	0.13

Table 2. Contd.

S/No	Compounds detected	CAS No.	Retention time	% Area
52	1,3-Bis-(2-cyclopropyl	1000222-08-6	22.533	0.79
53	Cetene, Oxalic acid, allyl hexadecyl ester	000629-73-2	22.691	1.06
54	Guaiol	000489-86-1	22.927	0.45
55	3-Cyclohexen-1-carboxaldehyde	1000131-99-4	23.181	0.11
56	Apiol, 1,3,6-Cyclooctatriene	000523-80-8	23.598	0.16
57	Aromandendrene,	000489-39-4	23.682	0.29
58	tau.-Muurolol, 1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a	019912-62-0	23.983	0.21
59	Naphthalene, 1,2,3,5,6,7,8,8a	004630-07-3	24.299	0.80
60	Alloaromadendrene	025246-27-9	24.607	0.23
61	octahydro-1,8a-dimethyl-7-(1-methylethenyl)	004630-07-3	24.711	0.35
62	1H-Cycloprop[e]azulene	028580-43-0	25.073	0.34
63	8a-dimethyl-7-(1-methylethenyl)	004630-07-3	25.259	0.34
64	1-Octadecene	000112-88-9	27.255	0.23
65	8-Hexadecenal	060609-53-2	27.790	0.10
66	Ethanone, 1-(4-methyl-1H-imidazol-2-yl)	002524-90-5	29.764	0.83
67	1,2-Benzenedicarboxylic acid	000085-69-8	30.016	0.54
68	3-Heptafluorobutyroxypentadecane	1000245-50-1	30.256	1.20
69	6-(Trifluoromethoxy)-N-(trimethylsilyl)	1000408-10-6	30.432	0.58
70	1-Octadecene	000112-88-9	31.081	0.43
71	Phytol	000150-86-7	31.306	0.15
72	Linoleic acid ethyl ester	000544-35-4	31.628	0.22
73	9-Oxabicyclo [6.1.0] nonane	000286-62-4	31.664	0.54
74	1-Docosene	001599-67-3	31.813	0.23
75	Trifluoroacetic acid	959010-23-2	32.966	0.29
76	(E)-5-(Benzo[d][1,3] dioxol-5-yl)	023512-53-0	33.264	1.18
77	Bis(2-ethylhexyl) phthalate	000117-81-7	34.004	0.34
78	(1S,15S)-Bicyclo[13.1.0]hexadecan-2-one	102572-89-4	34.141	0.30
79	Tetradecanoic acid,	056009-40-6	34.213	0.47
80	1-Piperonyl-3,5-diamino-1	031127-29-4	34.685	3.24
81	1-Piperonyl-3,5-diamino-1	031127-29-4	34.884	8.15
82	E-1,9-Hexadecadiene	1000245-71-4	35.655	0.41
83	Tricyclo[4.2.0.0(2,4)]octan-5-one	019004-82-1	35.842	1.23
84	Piperine	000094-62-2	36.007	0.68
85	Furo[3,4-c] pyridine-3,4(1H,5H)-dione	007472-18-6	36.183	0.89
86	Benzamide, N-(4-cyanomethylphenyl)	1000303-55-9	36.447	1.14
87	Pyrrolidine	025924-78-1	37.409	-0.15

volatile terpenoids play complementary and synergistic roles in the medicinal effects of *A. paniculata*.

Results obtained from the present study showed that Aromandendrene, is the most abundant compound identified in this study. Bakkali *et al.* (2008) reports on the use of *sesquiterpene* (Aromandendrene) has shown that it inhibits the growth of pathogenic bacteria and fungi, making it relevant for wound management, mastitis control, and skin infections in animals.

One of the major challenges faced by dairy farmers in Nigeria and West Africa at large is inflammatory conditions such as bovine mastitis and enteritis, which significantly reduce productivity. Aromandendrene-containing extracts may serve as natural alternatives to antibiotics, reducing

the risk of antimicrobial resistance while maintaining therapeutic efficacy.

Linalool is a monoterpene alcohol widely distributed in medicinal and aromatic plants. It is well documented for its antioxidant, anti-inflammatory, antimicrobial, cardioprotective, and neuroprotective activities (Mohamed *et al.*, 2021). Linalool exerts antioxidant effects by scavenging free radicals and modulating oxidative stress pathways, thereby protecting cellular membranes from lipid peroxidation (Peana *et al.*, 2002).

Pharmacological studies have shown that linalool can reduce inflammatory mediators such as nitric oxide (NO), prostaglandins, and pro-inflammatory cytokines, making it relevant in the management of inflammatory diseases

infections, and inflammatory conditions, and suggests that volatile constituents may enhance the overall therapeutic profile of the plant. The detection of Linalool in our *A. paniculata* extract aligns with the report of Ke *et al.* (2020) that this aromatic alcohol has cardiovascular potential. Linalool has demonstrated significant antihypertensive effects in SHR models through enhanced vasorelaxation and attenuation of cardiac hypertrophy. Additionally, it exerts cardioprotective effects against ischemia/reperfusion injury by reducing oxidative stress, inflammation, and cardiac biomarkers. Thus, linalool likely contributes to the overall cardiovascular therapeutic activity of *A. paniculata* observed in this study.

γ -Terpinene is a monoterpene hydrocarbon recognised for its strong antioxidant and anti-inflammatory properties. It acts primarily as a hydrogen donor, neutralising reactive oxygen species (ROS) and preventing oxidative damage to biomolecules (Ruberto and Baratta, 2000). In addition to its antioxidant activity, γ -terpinene exhibits antimicrobial effects against a wide range of bacterial and fungal pathogens, supporting its role in infection control (Soković *et al.*, 2010). Its identification in *A. paniculata* may partially explain the plant's documented antimicrobial and immunomodulating properties, especially in traditional remedies for respiratory and gastrointestinal infections (Ke *et al.*, 2020).

The use of γ -Elemene, a sesquiterpene, has been extensively studied for its anticancer, immunomodulatory, and anti-inflammatory activities. It has been shown to inhibit tumour cell proliferation, induce apoptosis, and suppress angiogenesis in various cancer models (Xu *et al.*, 2013).

Mechanistically, γ -elemene interferes with cell cycle progression and modulates signalling pathways such as PI3K/Akt and MAPK, which are critical in cancer development (Wang *et al.*, 2012; Rasul *et al.*, 2017). It also enhances immune responses by stimulating macrophage activity and regulating cytokine production. The detection of γ -elemene in *A. paniculata* suggests that volatile constituents may contribute to the anticancer and immunotherapeutic potential traditionally attributed to the plant, alongside andrographolide. Its discovery serves as an insight for pharmaceutical companies to have an alternative biological/natural source for γ -elemene anticancer drug formulation.

α -Copaene and structurally related sesquiterpenes such as isodene and α -farnesene derivatives are known for their anti-inflammatory, antioxidant, and antimicrobial properties (Ke *et al.*, 2020). These compounds contribute to membrane stabilisation, inhibition of lipid peroxidation, and modulation of inflammatory pathways (Da Silva *et al.*, 2012). Although present in moderate quantities, these sesquiterpenes may act synergistically with other volatile and non-volatile constituents, enhancing the pharmacological efficacy of *A. paniculata* extracts.

From the literature of previous studies, it can be inferred that the combination of linalool, γ -terpinene, γ -elemene,

aromandendrene, and α -copaene highlights the potential of *Andrographis paniculata* extracts as natural growth-promoting agents, immune enhancers, antioxidant feed additives, and phytochemical alternatives to antibiotics. This aligns with global efforts to reduce antibiotic use in animal production while maintaining animal health and productivity.

Conclusion

The medicinal value of *Andrographis paniculata* is increasingly understood as a result of multi-component synergy rather than the action of a single compound. While diterpenoid lactones dominate LC–MS profiles, GC–MS-detected terpenoids such as linalool, γ -elemene, and aromandendrene provide complementary antioxidant, antimicrobial, anti-inflammatory, anticancer, and cardioprotective effects. This synergistic interaction may explain why whole-plant extracts often demonstrate broader and more potent biological activities than isolated compounds. Incorporating GC–MS volatile fingerprints into quality control protocols may therefore improve the standardisation and therapeutic consistency of *A. paniculata*-based formulations.

New value added to knowledge

This study is very relevant in the field of ethnobotany and veterinary pharmacology because it identified major bioactive markers like Aromandendrene (6.12%) as the dominant sesquiterpene marker for this specific Nigerian variety. By identifying broad-spectrum antimicrobial compounds (e.g., Linalool and γ -Terpinene), the study provides a scientific basis for using *A. paniculata* as a natural growth promoter and an alternative to synthetic antibiotics in livestock. The findings therefore offer baseline fingerprint data that can be used for future quality control and to ensure the therapeutic consistency of herbal formulations.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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