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Assessment of the phytochemical properties and cytotoxicity of three medicinal plants used by pig farmers against African Swine Fever in Southwest Nigeria

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Abstract

The findings underscore the importance of region-sensitive strategies for integrating ethnoveterinary knowledge into contemporary livestock health programmes, as adoption patterns differ across the Southwestern states of Nigeria. Of the 12 plants being used, brimstone leaf, sunflower leaf and banana leaf ranked highest. Phytochemical screening revealed that, although the samples shared several major phytochemical groups, notable qualitative and quantitative differences were evident. Sunflower aqueous extract (SAE) contained additional bioactive compounds of potential therapeutic relevance, whereas sunflower methanol extract (SME) exhibited stronger cardiac glycoside activity, indicating distinct medicinal attributes. Similarly, brimstone aqueous extract (BAE) and brimstone methanol extract (BME) differed in phytochemical composition, with brimstone aqueous extract (BAE) showing greater abundance of phenols, tannins, anthraquinones, steroids, and cardiac glycosides, suggesting a broader pharmacological profile. Also, the enhanced phytochemical richness observed in banana aqueous extract (BAAE) relative to banana methanol extract (BAME) further indicated wider therapeutic potential, particularly in antioxidant, antimicrobial, and metabolic-modulating activities, consistent with trends reported for related botanical species. Variations in phytochemical detectability across extracts may be attributed to differences in extraction and processing procedures. In vitro cytotoxicity assessment using the MTT assay demonstrated a concentration-dependent reduction in cytotoxicity, with sunflower leaf ($8.4x10^{14}$) and brimstone leaf aqueous extracts (368.1381) exhibiting the most favourable biocompatibility profiles.

Keywords: *Phytochemical, MTT assay, extract toxicity, African swine fever*

Livestock in many rural and tribal communities hold significant economic and social value, symbolising wealth, cultural heritage, and status (Prasanna et al., 2025). Where veterinary access is limited, farmers often rely on plants and ethnoveterinary remedies, which, though not curative, may improve resilience and reduce secondary infections (Onarinde et al., 2024). Fasina et al. (2013) reported antiviral and virucidal activity of *Ancistrocladus uncinatus* against African swine fever virus, while Offiah et al. (2012) documented *Khaya senegalensis*, *Adansonia digitata*, *Vitex doniana*, *Terminalia*

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avicennioides, and *T. macroptera*, to treat diarrhoea by pastoralists in Nigeria. Local beliefs and practices remain central to ethnoveterinary knowledge in primary animal healthcare, illustrating the diverse role of medicinal plants (Yibrah, 2014; Mthi et al., 2023). A southwestern Nigeria survey on ethnoveterinary practices of pig farmers against African swine fever ranked three plants highest: *Tithonia diversifolia, Morinda lucida*, and *Musa paradisiaca*, hence their selection for phytochemical and cytotoxicity analysis.

Ahuchaogu et al. (2020) and Roggers et al. (2025) reported *Tithonia diversifolia* (*Asteraceae*) to contain terpenoids, phenolics, and flavonoids, with antimalarial, antioxidant, wound-healing, anti-inflammatory, anticancer, and antimicrobial properties. *Morinda lucida* was also reported to contain alkaloids, tannins, saponins, flavonoids, and vitamins A, E, and K, showing antibiotic, antiviral, antiplasmodial, antiparasitic, and trypanocidal activity (Asuzu et al., 1990; Sodimu et al., 2021), while Kumari et al. (2023) and Salama et al. (2020) reported *Musa paradisiaca* leaves to contain phenols, flavonoids, and tannins, with antimicrobial, antioxidant, and anticancer potential.

Materials and methods

Survey

In the purposive sampling method, 300 pig farmers from six states (Lagos, Ogun, Osun, Ekiti, and Oyo) in Southwestern Nigeria, which hosts the largest concentration of pig farmers, were interviewed on the use of ethnoveterinary plants on their farms. A structured questionnaire was used in gathering information on different types of plants used by farmers as therapeutic or curative interventions for ASF outbreaks.

The relative frequency of citation (RFC) was used to evaluate each plant identified for preventing and treating animal diseases, such as African swine fever (Ahmad et al., 2014), based on citations. Plants with the highest score were chosen to be analysed and tested.

Sample collection and phytochemical analysis

Fresh leaves of Brimstone tree, Banana, and Sunflower were collected from Osun and Oyo States, Nigeria. The plant materials were identified in the Botany Department of the University of Ibadan, Oyo State, Nigeria. The leaves were air-dried, ground into powder, and analysed. The qualitative analysis was carried out using standard procedures described by Sofowara (2008), while the quantitative phytochemical analysis was performed using the method described by Krishnaiah et al. *(*2009).

Test for tannin

0.1 g of the sample was extracted with 10 mL distilled water, filtered, and treated with 0.1% $FeCl_3$. A brownish-green or blue-black colouration indicated the presence of tannins.

Test for saponins

0.1 g of the sample was mixed with 10 mL distilled water and vigorously shaken. 3 drops of olive oil were added to the froth; the emulsion formed, indicating the presence of saponins.

Test for flavonoids

A few drops of 1% NH_3 solution were added to the aqueous extract. A yellow colouration that disappeared upon addition of concentrated H_2SO_4 indicated the presence of flavonoids.

Test for terpenoids

0.1 g of the sample was mixed with 2 mL chloroform, followed by careful addition of 3 ml concentrated H_2SO_4. A reddish-brown interface indicated the presence of terpenoids.

Test for glycosides

0.1 g of the sample was diluted in 5 mL water and treated with glacial acetic acid containing ferric chloride, followed by concentrated H_2SO_4. A brown ring at the interface indicated the presence of cardiac glycosides (cardenolides).

Test for alkaloids

0.1 g of the sample was extracted with acid alcohol, filtered, and treated with dilute ammonia and chloroform. The extract was tested with Mayer's and Dragendorff's reagents, where cream or reddish-brown precipitates indicated the presence of alkaloids.

Test for anthraquinones

0.1 g of the sample was boiled with 10 ml H_2SO_4, filtered, and extracted with chloroform. Dilute ammonia was added to the chloroform layer, and colour changes were observed to indicate the presence of anthraquinones.

Test for steroids

0.1 g of the sample was dissolved in chloroform, filtered, and treated with acetic anhydride and concentrated H_2SO_4. A green colouration indicated the presence of steroids.

Test for phenol

0.1 g of the sample was extracted with ethanol, filtered, and treated with ferric chloride. A blue-black colouration indicated the presence of phenols.

Determination of alkaloid content

2 g of the sample was extracted with 200 ml of 10% acetic acid in ethanol, filtered, concentrated, and precipitated with concentrated NH_4OH. The precipitate was collected, washed, dried, weighed, and used to calculate the percentage alkaloid content.

Determination of flavonoid content

Total flavonoid content was determined using the aluminium chloride colourimetric assay, with quercetin as the reference standard. Absorbance was measured at 510 nm using a UV/Visible spectrophotometer, and results were expressed as mg quercetin equivalent (QE) per gram of extract.

Determination of terpenoid content

2 g of the sample was extracted with 200 ml of 100% ethanol for 4 hours, filtered, and concentrated to one-quarter of its original volume. The concentrate was partitioned with petroleum ether, and the petroleum ether layer was collected, dried, and weighed to determine the percentage terpenoid content.

Determination of saponin content

2 g of the sample was extracted with 20% ethanol at 55°C for 4 hours, filtered, re-extracted, and concentrated. The extract was purified by partitioning with diethyl ether and n-butanol, washed with 5% NaCl, evaporated, dried to constant weight, and used to calculate the percentage saponin content.

Determination of tannin content

Tannin content was determined using the Folin Ciocalteu method with gallic acid as the reference standard. Absorbance was measured at 725 nm using a UV/Visible spectrophotometer, and tannin content was expressed as mg gallic acid equivalent (GAE) per gram of extract.

Determination of total phenol content

Total phenolic content was determined using the Folin Ciocalteu method, with gallic acid as the reference standard. Absorbance was measured colorimetrically at 765 nm, and results were expressed as mg gallic acid equivalent (GAE) per gram of dry extract.

Toxicity and ISO 10993-5 cytotoxicity testing criteria

Cytotoxicity was evaluated using the MTT assay, a colourimetric method that measures cell metabolic activity, viability, and proliferation. The assay

Table 1: Different plants named by farmers to prevent and treat African Swine Fever

States	Ethnoveterinary Plants											
	BT	BST	BGL	GG	GBG	M	CL	NL	PO	BL	BAL	SF
Ekiti	12.1	30.3	3.04	6.06	6.06	9.09	6.06	9.09	6.06	12.12	0	0
Lagos	12.5	31.25	3.13	6.25	3.13	9.36	6.25	9.38	6.25	12.5	0	0
Osun	0	0	0	0	0	0	0	0	0	0	0	100
Oyo	0	0	11.2	14.3	12.3	5.7	0	18.1	20.4	0	0	18
Ondo	0	0	0	0	0	0	0	0	0	0	0	100
Ogun	0	0	0	0	0	12.5	12.5	0	0	12.5	37.5	25

BT: Boundary tree, BST: Brimstone tree, BGL: Bitter gourd leaf, G: Ginger, GBG: Ginger blend and Garlic, M: Moringa, CL: Cassava leaves, NL: Neem leaves, PO: Palm oil, BL: Bitter leave, BAL: Banana leaf, SF: Sunflower

Table 2: Qualitative phytochemical screening of sunflower leaves, brimestone leaves, and banana leaves

Parameters	Sunflower Aqueous	Sunflower Methanol	Brimestone Aqueous	Brimestone Methanol	Banana Aqueous	Banana Methanol
Alkaloids	++Ve	++Ve	++Ve	++Ve	+Ve	+Ve
Flavonoids	+Ve	+Ve	++Ve	++Ve	++Ve	+Ve
Tannins	++Ve	++Ve	++Ve	+Ve	++Ve	+Ve
Saponins	++Ve	++Ve	+Ve	+Ve	++Ve	+Ve
Anthraquinones	+Ve	-Ve	++Ve	+Ve	+Ve	-Ve
Terpenoids	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Phenol	++Ve	+Ve	++Ve	+Ve	++Ve	+Ve
Steroids	++Ve	-Ve	++Ve	+Ve	+Ve	-Ve
Cardiac Glycosides	+Ve	++Ve	++Ve	+Ve	++Ve	++Ve

KEYS: +ve: present, ++ve: abundant, -ve: absent

is based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), a yellow tetrazolium salt, into insoluble purple formazan crystals by metabolically active cells. The quantity of formazan formed is directly proportional to the number of viable cells, allowing the determination of cell viability and the calculation of the IC_{50} value, defined as the concentration required to reduce cell viability by 50%. Cytotoxicity was interpreted according to the ISO 10993-5 guidelines for *in-vitro* biological evaluation of materials (ISO, 2022).

Result and discussion

This broad reliance on plant-based livestock health management supports previous reports that ethnoveterinary knowledge is locality-specific and shaped by farmers' beliefs, experiences, skills, and cultural practices (Yibrah, 2014). In contrast, Osun and Ondo demonstrated limited diversity, each reporting sunflower as the only dominant ethnoveterinary plant (99%), suggesting a specialized rather than diversified practice. Oyo recorded only trace utilization of Moringa, neem leaves, and sunflower (0.0019), indicating low documentation or limited dependence on these remedies. Ogun State displayed a distinct pattern characterized by high utilisation of Moringa, cassava leaf, banana, and sunflower, reflecting region-specific preferences for medicinal plants. These variations in state utilisation underscore the influence of cultural traditions, availability of medicinal plant resources, farmer knowledge, and local disease challenges on ethnoveterinary practices, consistent with (Onarinde et al. 2024; Mafimisebi 2012), who reported significant differences in the utilization of ethnoveterinary plants among Ondo, Oyo, and Ogun States, with Ogun recording the highest level of use.

Qualitative phytochemical screening of sunflower leaves aqueous (SAE) and methanol extractions (SME)

The qualitative phytochemical assessment of sunflower aqueous (SAE) and methanol (SME) extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids in both extracts, indicating similar core secondary metabolite profiles, consistent with the reports of Ngibad (2019) and Fashogbon (2025). These phytochemicals are recognized for their antioxidant, antimicrobial, anti-inflammatory, and therapeutic activities. However, anthraquinones and steroids were detected only in SAE, consistent with Christina et al. (2017), suggesting additional antimicrobial, anti-inflammatory, and hormone-modulatory properties. In contrast, phenolic compounds were more abundant in SAE (++ve) than in SME (+ve), indicating a potentially higher antioxidant capacity. In contrast, cardiac glycosides were more strongly expressed in SME (++ve) than in SAE (+ve), suggesting enhanced cardiotonic potential characteristic of this class of bioactive compounds (Tasneem, 2015).

Qualitative phytochemical screening of brimstone leaves aqueous (BAE), and methanol extractions (BME)

Phytochemical analysis of brimstone aqueous (BAE) and methanol (BME) extracts revealed the presence of alkaloids, flavonoids, saponins, terpenoids, phenols, steroids, and cardiac glycosides in both extracts, with BAE exhibiting stronger reactions for tannins, anthraquinones, phenols, steroids, and cardiac glycosides (++), compared with the moderate levels (+) observed in BME. Alkaloids and flavonoids were strongly present in both extracts, while saponins and terpenoids showed moderate abundance. The occurrence of these bioactive compounds is consistent with previous reports on medicinal plants rich in secondary metabolites, with flavonoids and phenolic compounds contributing antioxidant, anti-inflammatory, and antimicrobial activities (Kumar & Pandey, 2013), alkaloids exhibiting analgesic, antimicrobial and anticancer properties (Sarker & Nahar, 2017), and terpenoids and saponins providing anti-inflammatory, immunomodulatory, and antimicrobial effects (Del Prado-Audelo et al., 2021; Timilsena et al., 2023). The higher anthraquinone content in BAE suggested enhanced antimicrobial and cytotoxic potential, as reported for compounds such as emodin and aloe-emodin (Zhao et al., 2023), while the stronger presence of steroids and cardiac glycosides indicated possible lipid-modulating, anti-inflammatory, and cardiotonic activities, although the latter require careful safety evaluation due to their narrow therapeutic indices (Botelho et al., 2019).

Qualitative phytochemical screening of banana leaves aqueous (BAAE) and methanol extractions (BAME)

Table 3: Quantitative phytochemical screening of sunflower leaves, brimstone leaves, and banana leaves

Parameters	Sunflower Aqueous	Sunflower Methanol	Brimestone Aqueous	Brimestone Methanol	Banana Aqueous	Banana Methanol
Alkaloids	2.20±0.004	2.45±0.001	2.10±0.003	3.20±0.001	1.35±0.004	1.55±0.001
Flavonoids	0.18±0.358	3.28±0.126	1.07±0.086	1.77±0.024	0.26±0.133	1.54±0.133
Tannins	1.82±0.001	1.96±0.000	1.15±0.005	1.29±0.010	1.06±0.001	1.14±0.005
Saponins	1.50±0.004	1.45±0.002	1.50±0.002	1.00±0.001	1.30±0.004	2.90±0.002
Terpenoids	0.50±0.001	1.50±0.003	0.55±0.002	1.90±0.004	0.30±0.001	0.70±0.003
Phenol	0.85±0.004	1.17±0.001	0.93±0.002	0.96±0.001	0.73±0.006	0.82±0.008

Banana leaf aqueous (BAAE) and methanol (BAME) extracts showed the presence of alkaloids, terpenoids, cardiac glycosides, steroids, saponins, flavonoids, tannins, and anthraquinones in varying concentrations, confirming banana leaves as a rich source of bioactive compounds with significant medicinal potential (Choudhury et al., 2023). Cardiac glycosides, phenols, saponins, tannins, and flavonoids were more abundant in BAAE, while cardiac glycosides are predominant in BAME. The detection of flavonoids and tannins in BAME contradicted the findings of Bashir et al. (2025), who reported their absence but observed steroids, which were not detected in the present methanol extract. The occurrence of phenols and terpenoids supported earlier reports of their antioxidant and antimicrobial activities (Kumar & Pandey, 2013; Cushnie & Lamb, 2011), while the higher levels of flavonoids, phenols, tannins, and saponins in BAAE suggested enhanced antioxidant, anti-inflammatory, antidiarrheal, hypoglycaemic, and hypocholesterolaemic properties (Sharma et al., 2019; Sharma et al., 2023). Anthraquinones and steroids, detected only in BAAE, may confer additional antimicrobial, anticancer, laxative, and anti-inflammatory effects (Zhao et al., 2023; Li et al., 2024). The strong presence of cardiac glycosides in both extracts agreed with previous reports of their cardiotonic activity through Na^+/K^+-ATPase inhibition (Botelho et al., 2019). However, Widoyanti (2023), reported the absence of tannins and cardiac glycosides in aqueous banana leaf extracts.

Quantitative phytochemical screening of sunflower leaves in aqueous (SAE) and methanol extraction (SME)

Phytochemical screening and quantification of two extraction methods of sunflower samples revealed differences in secondary metabolite composition. SME exhibited higher mean levels of alkaloids, flavonoids, tannins, terpenoids, and phenols compared with SAE, whereas saponins were slightly lower. The most pronounced differences were observed for flavonoids and terpenoids; however, the flavonoid measurement for SAE displayed a large relative standard deviation (0.16 ± 0.35), indicating substantial assay variability or values close to the detection limit. These observed compositional differences may reflect genetic, developmental, environmental, or methodological factors and warrant further confirmatory analysis (e.g., HPLC quantification and statistical testing). Variations in phytochemicals' detectability may be due to the processing procedure used in extraction, which may have resulted in increased or reduced detectability (Christina et al. 2017).

Quantitative phytochemical screening of brimstone leaves in aqueous (BAE) and methanol extraction(BME)

The analysis showed notable differences between brimstone aqueous extract (BAE) and brimstone methanol extract (BME), with BME containing significantly higher concentrations of alkaloids (3.20 ± 0.001), terpenoids (1.90 ± 0.004), flavonoids (1.77 ± 0.024), and tannins (1.29 ± 0.010), indicating a richer bioactive profile with enhanced antimicrobial, antioxidant, anti-inflammatory, and pharmacological potential. These findings are consistent with Sodimu (2021), who reported that *Morinda lucida* leaves possess high levels of flavonoids, steroids, tannins, and phenols. In contrast, BAE exhibited a substantially higher saponin content (1.50 ± 0.002), suggesting greater cholesterol-lowering, immunomodulatory, and membrane-stabilizing properties, as reported by Suradkar et al. (2017), who attributed the medicinal applications of *M. lucida* in West African traditional medicine to the presence of saponins and cardiac glycosides. The phenolic content was comparable between the two extracts (0.93 ± 0.001 for BAE and 0.96 ± 0.001 for BME), indicating similar phenol-mediated antioxidant capacities.

Quantitative phytochemical screening of banana leaves in aqueous (BAAE) and methanol extractions (BAME)

Quantitative screening showed that banana methanol extract (BAME) contained higher concentrations of all measured phytochemicals than banana aqueous extract (BAAE), indicating a superior bioactive profile. BAME recorded higher alkaloid (1.55 ± 0.001 vs. 1.35 ± 0.004), flavonoid (1.54 ± 0.133 vs. 0.26 ± 0.133), tannin (1.14 ± 0.005), saponin (2.90 ± 0.002), terpenoid (0.70 ± 0.003), and phenolic (0.82 ± 0.008 vs. 0.73 ± 0.006) contents than BAAE. The elevated flavonoid and phenolic concentrations suggested greater antioxidant and anti-inflammatory activities, while the higher saponin and terpenoid levels indicate enhanced immunomodulatory,

Table 4: Cytotoxicity test for aqueous and methanol extracted test leaves

Test material	0µL (Neat)	Dilution 1 (-1µL)	Dilution 2 (-2µL)	Dilution 3 (-3µL)	Cell viability index
Brimstone Methanol	48.87	57.87	76.63	80.92	0.904129
Brimstone Aqueous	75.35	72.99	89.5	83.49	368.1381
Sunflower Methanol	0	51.55	92.82	109.97	0.445234
Sunflower Aqueous	104.39	107.07	108.25	109.33	$8.4x10^{14}$
Banana Methanol	0	65.27	76.85	77.7	1.145232
Banana aqueous	50.48	0	109.22	119.51	2.164298

cholesterol-lowering, and pharmacological properties. These findings agreed with Widoyanti (2023), who associated high phenolic and flavonoid contents with increased antioxidant potential, and with Gaafar et al. (2015), who reported that the higher trolox equivalent antioxidant capacity (TEAC) of banana methanol extract is directly related to its greater phenolic content.

In vitro toxicity test of test plants

Cytotoxicity assessment revealed that the methanol extracts of brimstone, sunflower, and banana leaves exhibited concentration-dependent effects, with neat or higher concentrations showing reduced cell viability that improved upon dilution. Brimstone methanol extract recorded 48.86% viability at 100% concentration but increased to 76.62% and 80.91% at –2µL and –3µL dilutions, while sunflower methanol extract improved from 51.54% at –1 to 92.81% and 109.96% at lower concentrations. Similarly, banana methanol extract showed slight cytotoxicity at –1µL (65.26%) but achieved non-cytotoxic responses (≥70%) at –2µL and –3µL dilutions. In contrast, brimstone aqueous extract maintain cell viability above the ISO 10993-5 non-cytotoxic threshold (75.35–89.40%) across all concentrations, sunflower aqueous extract consistently exceeded 100% viability, indicating possible stimulation of cell metabolic activity, and banana aqueous extract showed slight cytotoxicity at –1µL (50.47%) but non-cytotoxic and proliferative responses at –2µL and –3µL (109.21% and 119.50%). These findings demonstrated that aqueous extracts are generally safer than methanol extracts and that cytotoxicity is dose-dependent, consistent with the reports of Oyesola et al. (2010), Taziebou et al. (2007), and Irvine (2020).

Conclusion

The study demonstrated that ethnoveterinary plant utilisation varies considerably across Southwestern Nigeria, emphasizing the need for region-specific integration of indigenous knowledge into modern livestock health systems. Although the leaf extracts shared similar phytochemical classes, differences in composition and activity revealed distinct therapeutic potentials, with sunflower aqueous extract (SAE) containing additional bioactive compounds, sunflower methanol extract (SME) exhibiting stronger cardiac glycoside activity, and brimstone and banana extracts showing varying phytochemical richness depending on the extraction method. The higher concentrations of phenols, tannins, anthraquinones, steroids, and cardiac glycosides in aqueous extracts suggest enhanced antioxidant, antimicrobial, and metabolic-modulating properties, while BME and BAME displayed greater overall phytochemical diversity and BAE showed superior saponin activity. Furthermore, the MTT assay confirmed that cytotoxicity was concentration dependent, with sunflower and brimstone aqueous extracts exhibiting the best in vitro biocompatibility.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Conflict of interest

The authors declare no conflict of interest

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