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Phytochemical Screening, FT-IR and GC-MS Analysis of Extracts from *Aloe Aculeata* Pole-Evans Leaves used for Managing Poultry Health in Drylands of Zimbabwe

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Abstract: Due to limited veterinary facilities, farmers in remote areas of Zimbabwe rely heavily on herbal ethnoveterinary medicines as a feasible alternative to conventional veterinary operations. Medicinal plants may be the best prospects for developing drugs to treat animal health problems. The medicinal properties of medicinal plants are attributable to the existence of diverse bioactive compounds with varying compositions that arise as secondary metabolites. According to research conducted in the drylands of Zimbabwe's Masvingo province, *Aloe aculeata* was the most commonly utilized Aloe species for managing poultry health. However, the scientific basis for the curative properties of *Aloe aculeata* had not yet been established. As a result, this study was done to address the aforementioned knowledge gap with the objective of performing a preliminary investigation of potential phytochemicals and to identify and characterize potential phytochemicals using FT-IR analysis and the GC-MS technique. Tannins, phenolic compounds, flavonoids, and phlobatannins tests gave positive results across extracts from shade-dried and fresh leaves from all three solvents (hexane, distilled water, ethanol). Flavonoids had the highest concentration in the leaf samples (5.6%), followed by alkaloids (3.2%) and saponins (3.15%). FT-IR analysis confirmed the presence of phenols, amines, aromatics, alkynes, alkenes, and quinones due to the functional groups that were detected. GC-MS analysis showed the presence of 32 compounds, and 17 have reported pharmacological activity. The high number of phytochemicals and compounds with pharmaceutical value justifies the use of Aloe aculeata in managing poultry diseases. The findings of the present study offer the ethnomedical use of this plant for the development of herbal drugs for the management of poultry health.

Keywords: *Aloe aculeata*; poultry; drylands; ethnoveterinary; phytochemical; drug development

1. Introduction

Due to limited veterinary facilities, farmers in remote areas of Zimbabwe rely heavily on herbal plants as a feasible alternative to conventional veterinary medicines (Sibanda & Chiuta, 2018). EVM studies are significant since plants contain a wide range of phytochemicals. Medicinal plants may be the best prospects for developing drugs and other active chemicals to treat human and cattle health problems due to their phytochemical composition (Shin & Park, 2018).

Medicinal plants contain chemical compounds called phytochemicals that can affect the body's physiological processes, helping to treat or prevent diseases in humans and animals (Gurib-fakim, 2006). Medicinal properties of herbal plants are attributable to diverse bioactive compounds with varying compositions that arise as secondary metabolites (Twaij & Hasan, 2022). Bioactive compounds are natural chemicals in plants, animals, and microorganisms that have biological effects on living tissues. They often contribute to the medicinal properties of ethnoveterinary remedies and herbal medicines (Balsano & Alisi, 2009). Bioactive compounds have biological effects on living matter. Saponins, essential oils, glycosides, phytosterols, tannins, flavonoids, terpenoids, and alkaloids are bioactive compounds (Jaiswal et al., 2020). Bioactive natural products are molecules refined by evolution that, based on their physicochemical attributes, are more likely to become viable therapeutic candidates than synthetic compounds created using combinatorial chemical processes (Lahlou, 2013).

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Aloe aculeata Pole-Evans is a species of Aloe native to South Africa and parts of southern and central Zimbabwe and Mozambique (Mapunya et al., 2012; Pole-evans, 1919). Aloe aculeata is a succulent, stemless Aloe species that forms a compact, stemless rosette of leaves with scattered, reddish-brown spines (Pole-evans, 1919). In the earlier studies of this research, Aloe aculeata was the most commonly utilized Aloe species for managing poultry health (Gobvu et al., 2024). Aloe species are commended for their curative properties (Egbuna et al., 2020), however the scientific basis for these actions in Aloe aculeata has not yet been established. As a result, this study was done to address the aforementioned knowledge gap. The objective was to perform a preliminary investigation of potential phytochemicals, to quantify the phytochemicals, and to identify and characterize potential phytochemicals with the application of GC-MS and FT-IR assays.

2. Materials and methods

2.1 Species identification

Leaf samples of A. aculeata were randomly collected from various locations in May 2023 and authenticated at the National Herbarium and Botanical Gardens in Harare, Zimbabwe. Samples were collected in a way to avoid plants that were dead or insect-damaged and mechanically injured. The plant was identified with the assistance of local people. Field notes were taken, which included the village and ward from, landforms close to, types of rocks and soils in the area, date, and time of collection. Specimens were pressed in the field and dried to avoid fungal attack while retaining their colour and arrangement using a 45 by 30 cm plant press. The A. aculeata leaf samples were deposited as Specimen Voucher GOBVU V3 at the National Herbarium and Botanical Garden in Harare, Zimbabwe.

2.2 Collection of plant material

Fresh leaves of Aloe aculeata Pole-Evans were collected from Denga village under ward 8 in Chivi district of Masvingo province, Zimbabwe. A knife was used to cut off the leaves to be used for laboratory analysis.

2.3 Dried leaves sample extraction

Leaves of Aloe aculeata Pole-Evans were washed with water, after which they were dried in the shade for four weeks. Shade drying was done to evade direct loss of phytoconstituents from sunlight. The dried leaves were ground into powder using a traditional mortar and pestle. Coarse particles were filtered out with a 2 mm sieve, and the resulting fine powder was stored in airtight containers. The maceration technique was used as the extraction method. 100g of powdered samples were placed in containers. Three different solvents were used for extraction and these are distilled water, absolute ethanol, and hexane. The menstruum was poured on top until it completely covered the ground A. aculeata powder. The containers were closed with parafilm for 48 hours. The contents were stirred periodically to maximize extraction. After extraction, the extracts were filtered through Whatman No. 1 filter paper. The filtrate was placed in the oven at 40 °C to separate it from the solvent. The crude extract was left to dry until it reached a constant weight.

2.4 Extraction of fresh leaves

Aloe aculeata leaves were thoroughly washed using distilled water. Leaves were dissected longitudinally, and the rind was separated from the inner fillet (gel) with a knife. The rind and the inner gel were weighed separately. Using the maceration process described in section 2.3, 200g of fresh rind and fresh gel were placed in containers, and different solvents were added.

2.5 Comparison of extraction yield in various solvent extracts of Aloe aculeata

The extraction yield percentage for each solvent extract of A. aculeata leaves was determined using the formula:

$$\text{Extraction yield (\%)} = (m1 / m0) \times 100$$

where m1 represents the total mass of the crude extract (g), and m0 is the initial mass of the plant material used for extraction.

2.6 Qualitative phytochemical analysis

Phytochemical analysis of the ethanol, distilled water, and hexane extracts of both dried and fresh leaves was undertaken using methods as described by Gariba et al. (2021).

2.6.1 Test for Alkaloids (Mayer's Test)

The extracts were dissolved in Dragendorff's reagent to prepare 1 mL of extract solution. Then, 1 mL of Mayer's reagent was added to this solution. The appearance of a whitish-yellow or cream-coloured precipitate indicated the presence of alkaloids.

2.6.2 Test for Steroids

Half a millilitre of the extract solution was mixed with chloroform in a test tube. A few drops of concentrated sulfuric acid were added, and the mixture was shaken thoroughly. The formation of a red colour at the lower layer indicated the presence of steroids, while the appearance of a yellow layer suggested the presence of terpenoids and steroids.

2.6.3 Test for Terpenoids

Half a millilitre of the extract solution was mixed with chloroform in a test tube. A few drops of concentrated sulfuric acid were added, and the mixture was shaken well. The appearance of a reddish-brown colour indicated the presence of terpenoids, while a yellow layer suggested the presence of both terpenoids and steroids.

2.6.4 Test for Flavonoids

One millilitre of the extract solution was combined with four small pieces of magnesium ribbon, followed by the dropwise addition of concentrated hydrochloric acid. A pink to scarlet colour developing

Fig. 1. Separation of gel (inner fillet) from the rind (outer layer) of Aloe aculeata leaves

after a few minutes indicated the presence of flavonoids.

2.6.5 Test for Saponins

Half a gram of each plant extract was shaken with water in a test tube. A few drops of olive oil were added, and the mixture was shaken vigorously. The formation of a stable emulsion indicated the presence of saponins.

2.6.6 Test for Tannins and Phenolic Compounds

A few drops of ferric chloride were added to 0.5 mL of the extract solution. The appearance of a blue-green colour indicated the presence of tannins and phenolic compounds.

2.6.7 Test for Coumarins

One millilitre of the extract solution was mixed with 1.5 mL of 10% sodium hydroxide (NaOH). The appearance of a yellow colour indicated the presence of coumarins.

2.6.8 Test for Anthraquinones

Half a gram of the plant extract was shaken with 5 mL of benzene, then filtered. To the filtrate, 5 mL of 10% ammonia solution was added, and the mixture was shaken. The appearance of a pink-red or violet colour indicated the presence of anthraquinones.

2.6.9 Test for phlobatannins (HCl test)

Two millilitres of extract solution were mixed with dilute hydrochloric acid (HCl). The formation of a red precipitate indicated the presence of phlobatannins.

2.7 Quantitative analysis of phytochemicals in Aloe aculeata leaves

Alkaloids (Harbone, 1973)

Alkaloids were quantified by mixing 5 g of Aloe aculeata leaf powder with 200 mL of 10% acetic acid in ethanol. The mixture was covered and left to stand for four hours. The filtrate was then concentrated to one-fourth of its original volume using a water bath. Concentrated ammonium hydroxide was added dropwise to the extract until precipitation occurred. The solution was allowed to settle completely, and the precipitates were collected, washed with diluted ammonium hydroxide, filtered, dried, and then weighed.

Flavonoids (Bohm & Koupai-Abyazani, 1994)

Ten grams of powdered plant material were extracted twice using 10 mL of 80% aqueous methanol at room temperature. The solution was then filtered through Whatman No. 1 filter paper, and the filtrate was placed in porcelain crucibles and dried on a water bath until a constant weight was achieved.

Saponins (Obadoni & Ochuko, 2002)

To quantitatively determine saponins, 20 g of powdered sample was mixed with 100 mL of 20% aqueous ethanol and shaken for 30 minutes. The mixture was then heated in a water bath at 55 °C for 4 hours. After filtration, the residue was re-extracted with 200 mL of 20% aqueous ethanol. The combined extracts were concentrated to about 40 mL over a water bath at 90°C. The concentrate was transferred into a 250 mL separating funnel and extracted twice with 20 mL of diethyl ether. The aqueous layer was retained while the ether layer was discarded. Next, 60 mL of n -butanol was added to the aqueous layer, and the n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The final solution was heated in a water bath to evaporate the solvent, then dried in an oven at 40°C to a constant weight. The saponin content was expressed as a percentage of the initial dry weight of the sample.

2.8 FT-IR Spectroscopic Analysis of shade-dried *A. aculeata* leaves

Fourier Transform Infrared (FT-IR) spectroscopy analysis was used to detect functional groups of extracts from *A. aculeata* leaves. Dried powder from *A. aculeata* leaves was used for FT-IR analysis. Ten milligrams of the dried leaf powder were mixed with 100 mg of KBr to form translucent sample pellets. Each powdered sample was then placed in an FT-IR spectroscope (Shimadzu, IR Affinity 1, Japan) and scanned over a range of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The analysis was conducted using Omnic software (version 5.2).

2.9 GC-MS Analysis

A. aculeata leaf powder was dissolved and extracted using HPLC-grade methanol, then filtered through 0.45 µm syringe filters. The resulting extract was placed in auto-sampler vials for GC-MS analysis (Shimadzu QP 2010). Ultrapure helium served as the carrier gas at a flow rate of 1 mL/min. Separation was achieved on a 30-meter BPX5 non-polar column (0.25 mm ID, 0.25 µm film thickness). The GC-MS oven was programmed to start at 60°C (held for 1 minute), then ramped at 10°C/min to 270°C over 18 minutes, totaling a 30-minute runtime. One microliter of sample was injected in split mode (split ratio 10:1) at 200°C, with the interface temperature at 250°C and the electron ionization source set at 200°C. Mass spectra were recorded in full scan mode over 50 –500 m/z.

Peak identification was performed by auto-matching spectra against the NIST library (over 62,000 patterns), using TurboMass 5.2 software (2005). Each compound's name, molecular weight, and structure were determined, and relative percentages were calculated based on peak area proportions. This analysis aimed to identify compounds in A. aculeata leaf extract that may justify its traditional use in poultry health management.

3. Results

3.1 Comparison of extraction yield in various solvent extracts of *Aloe aculeata*

High yield was recorded for dried samples more to the fresh samples. Overall, distilled water showed the best extraction ability for all the samples, while hexane showed poor extraction ability for the Aloe aculeata samples (**Table 1**). For the shade dried leaves, distilled water had the highest extraction yield (5.05%) while hexane had the least extraction yield (0.75%). For the gel from fresh leaves, distilled water had the highest extraction yield (1.05%), and hexane had the lowest extraction yield (0.3125%). Ethanol had the highest extraction yields for fresh rind (4.6%), followed by distilled water (4.53%) while hexane had the least extraction yield (0.0045%).

3.2 Qualitative phytochemical analysis of Aloe aculeata extracts

The present study carried out on Aloe aculeata revealed the presence of bioactive compounds in the plant species. The phytochemical active compounds of Aloe aculeata were qualitatively analyzed, and the results are presented in **Table 2** and **3**.

Alkaloids were present only in distilled water extracts from shade-dried leaves and fresh gel. Tannins, phenolic compounds, flavonoids and phlobatannins tests gave positive results across extracts from shade-dried leaves from all three solvents (**Table 2**). In shade-dried leaves, anthraquinones were only present in ethanol extracts while saponins were only detected in distilled water extracts (**Table 2**).

Table 1: Extraction yields for *A. aculeata* leaves

Sample	Solvent	Initial sample weight (g)	Extract weight (g)	Yield (%)
Dried leaves	Distilled water	100	5.050	5.0500
	Ethanol	100	2.810	2.8100
	Hexane	100	0.750	0.7500
Fresh gel	Distilled water	200	2.106	1.0530
	Ethanol	200	1.116	0.5580
	Hexane	200	0.625	0.3125
Fresh rind	Distilled water	200	9.053	4.5265
	Ethanol	200	9.218	4.6090
	Hexane	200	0.009	0.0045

In screening fresh leaves, tannins, phenolic compounds, steroids, terpenoids, flavonoids and phlobatannins tests gave positive results across all solvents in both fresh gel and fresh rind (**Table 3**). Except for saponins and alkaloids, all other phytochemicals were present in all fresh gel extracts from ethanol and distilled water (**Table 3**). Saponins were detected only in distilled water extracts of fresh rind. Tests for alkaloids and coumarins gave negative results in all extracts from fresh rind.

3.3 Quantification of phytochemicals

The results of the quantification of phytochemicals in *Aloe aculeata* are shown in **Table 4.** Flavonoids had the highest concentration (5.6%) followed by alkaloids (3.2%) and saponins (3.15%) (**Table 4**).

3.4 FT-IR Peak Values and Functional Groups of shade-dried whole leaves powder of *Aloe aculeata*

FT-IR analysis of shade-dried A. aculeata leaves indicated the presence of phenols, amines, aromatics, alkynes, alkenes, quinones, and aliphatic organohalogen compounds, and the major peaks were at 3430.53,1615.97, 1454.09, 1127.03, 883.09 and 624.22 (**Figure 2**; **Table 5**). FT-IR analysis of shade-dried *A. aculeata* leaves indicated the presence of phenols, amines, aromatics, alkynes, alkenes, quinones, and aliphatic organohalogen compounds, and the major

Table 2: Qualitative phytochemical analysis of extracts from shade-dried whole *A. Aculeata* leaves. (+) indicates the presence of the phytochemical and (–) indicates the absence of the phytochemical.

Sample	Phytochemical	Plant extract		
		Hexane	Ethanol	Distilled water
Shade dried whole leaves	Saponins	-	-	+
	Alkaloids	-	-	+
	Tannins	+	+	+
	Phenolic Compounds	+	+	+
	Anthraquinones	-	+	-
	Steroids	+	-	+
	Terpenoids	-	+	-
	Flavonoids	+	+	+
	Phlobatannins	+	+	-
	Coumarins	-	+	+

peaks were at 3430.53,1615.97, 1454.09, 1127.03, 883.09 and 624.22 (**Figure 2**; **Table 5**).

Table 3: Qualitative phytochemical analysis of extracts from fresh leaves of *A. aculeata*. (+) indicates the presence of the phytochemical and (–) indicates the absence of the phytochemical.

Sample	Phytochemical	Extract	
		Ethanol	Distilled water
Fresh gel	Saponins	-	-
	Alkaloids	-	+
	Tannins	+	+
	Phenolic compounds	+	+
	Anthraquinones	+	+
	Steroids	+	+
	Terpenoids	+	+
	Flavonoids	+	+
	Phlobatannins	+	+
	Coumarins	+	+
Fresh rind	Saponins	-	+
	Alkaloids	-	-
	Tannins	+	+
	Phenolic compounds	+	+
	Anthraquinones	-	+
	Steroids	+	+
	Terpenoids	+	+
	Flavonoids	+	+
	Phlobatannins	+	+
	Coumarins	-	-

Table 4: Quantitative determination of alkaloids, flavonoids, and saponins in Aloe aculeata shade dried whole leaves. The yield is expressed as the weight of dried filtrate divided by the initial weight of the sample and expressed as a percentage.

Phytochemical	Initial weight of sample (g)	Weight of dried filtrate (g)	Yield (%)
Alkaloids	5	0.16	3.20
Flavonoids	10	0.56	5.60
Saponins	20	0.63	3.15

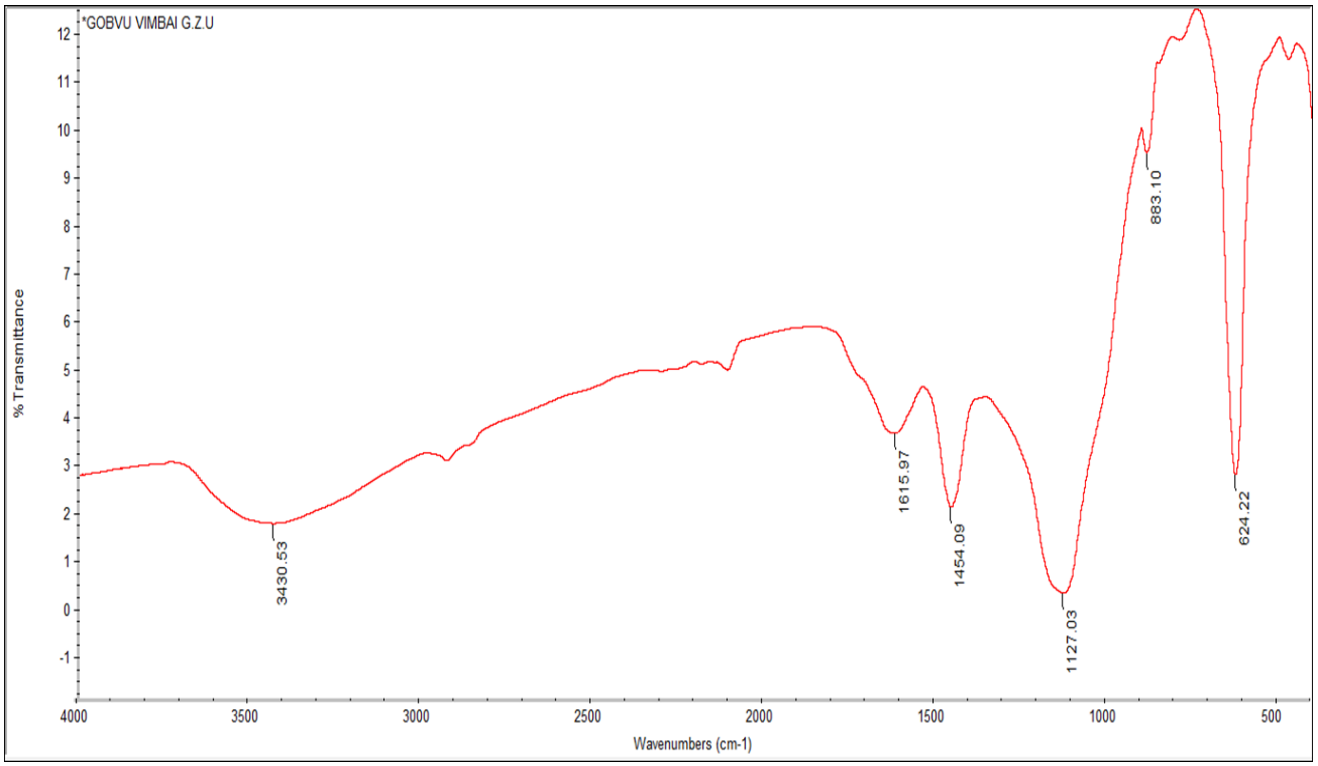

Figure 2: FT-IR peak values of Aloe aculeata shade-dried

Table 5: FT-IR Peak values, bands, corresponding functional groups and possible compounds identified in the shade-dried leaves of *Aloe aculeata*. The functional groups and frequencies are according to Nandiyanto et al., (2019)

Wavenumber cm-1						
Band range (Experimental)	**Band range (Literature)** (Nandiyanto et al., 2019)	**Band number**	**Band interaction**	**Band assignment**	**Functional group**	**Possible compound(s)**
3430.53	3570-3200	1	Stretch	O-H	Hydroxy	Alcohols Phenols
1615.97	1650-1590	2	Bend	N-H	Primary amino	Primary amine
	1650-1550		Bend	>N-H	Secondary amino	Secondary amine
	(1650-1600)[2]		Stretch	C=O	Carbonyl compound	Quinone or conjugated ketone
1454.09	1470-1430	3	Bend	C-H	Alkene/alkyl	Methyl
	1485-1445			>CH2		Methylene
1127.03	(1200-1100)[4]	4			Simple hetero-oxy compounds (sulfur oxy compounds)	Sulfonates
883.1	900-670	5	Bend (out of plane)	C-H	Aromatic ring (aryl)	Aromatics
624.22	680-610	6	Bend	C-H	Alkyne	Acetylene

Table 6: GC-MS analysis of phytocompounds identified in the methanolic extract of *Aloe aculeata* leaves. The component's retention time is the amount of time a compound spends on the column after it has been injected. The CAS number (CAS #) is a distinctive numerical identifier assigned by the Chemical Abstracts Service to each chemical substance. The CAS # ensures precise identification of chemicals across databases and publications.

Component RT	Compound Name	CAS#	Formula	Area	Match Score
3.2283	Nitric acid, 1-methylethyl ester	1712-64-7	C3H7NO3	28360	91.5
3.7295	2-Ethyl-1-hexanol	2000411-44-8	C8H18O	422188	92.2
4.3759	Phenylethyl Alcohol	60-12-8	C8H10O	107211	90.7
5.1111	Benzofuran, 2,3-dihydro-	496-16-2	C8H8O	12231410	93.8
5.4039	Nonanoic acid	112-05-0	C9H18O2	417310	92.6
5.9714	2-Methoxy-4-vinylphenol	7786-61-0	C9H10O2	637137	94.7
6.4365	Cyclopentasiloxane, decamethyl-	541-02-6	C10H30O5Si5	7277	91.2
8.0322	2,6-Difluorobenzoic acid, 4-nitrophenyl ester	2000307-56-2	C13H7F2NO4	55175	90.9
8.0436	Naphthalene, decahydro-4a-methyl-1-(4a.alpha.,7.alpha.,8a.beta.)]-	17066-67-0	C15H24	490585	95.5
8.0745	.beta.-D-Glucopyranose, 1,6-anhydro-	498-07-7	C6H10O5	10238746	90.8
8.7715	Dodecanoic acid	143-07-7	C12H24O2	444007	96.3
9.7289	2-Dimethylaminobenzoic acid	610-16-2	C9H11NO2	3270414	98.2
10.5651	3-Butylisobenzofuran-1(3H)-one	6066-49-5	C12H14O2	268829	95.5
11.0290	2,6-Diisopropylnaphthalene	24157-81-1	C16H20	112863	91.9
11.7330	2,6-Diisopropylnaphthalene	24157-81-1	C16H20	165052	91.9
11.8693	2,6-Diisopropylnaphthalene	24157-81-1	C16H20	84758	90.3
12.2903	Tetradecanoic acid	544-63-8	C14H28O2	1045800	98.5
12.6642	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-	73410-02-3	C11H16O3	312412	92.9
13.9164	Neophytadiene	504-96-1	C20H38	468160	96.7
14.0432	2-Pentadecanone, 6,10,14-trimethyl-	502-69-2	C18H36O	319566	97.0
16.5276	n-Hexadecanoic acid	57-10-3	C16H32O2	23383868	98.2
16.7438	Tetradecanamide	638-58-4	C14H29NO	696527	90.9
18.8208	Heptadecanoic acid	506-12-7	C17H34O2	592584	93.8
19.9838	Phytol	150-86-7	C20H40O	792267	98.0
20.3793	9,12-Octadecadienoic acid (Z,Z)-	60-33-3	C18H32O2	11460969	97.9
20.4953	cis-Vaccenic acid	506-17-2	C18H34O2	19279272	97.8
20.8565	Octadecanoic acid	57-11-4	C18H36O2	4094339	97.0
21.1219	Hexadecanamide	629-54-9	C16H33NO	2223784	91.3
22.8308	1,8,9-Anthracenetriol, 3-methyl-	491-59-8	C15H12O3	6107883	95.0
23.1265	9,10-Anthracenedione, 1,8-dihydroxy-3-methyl-	481-74-3	C15H10O4	2438884	97.0
3.2283	Nitric acid, 1-methylethyl ester	1712-64-7	C3H7NO3	28360	91.5

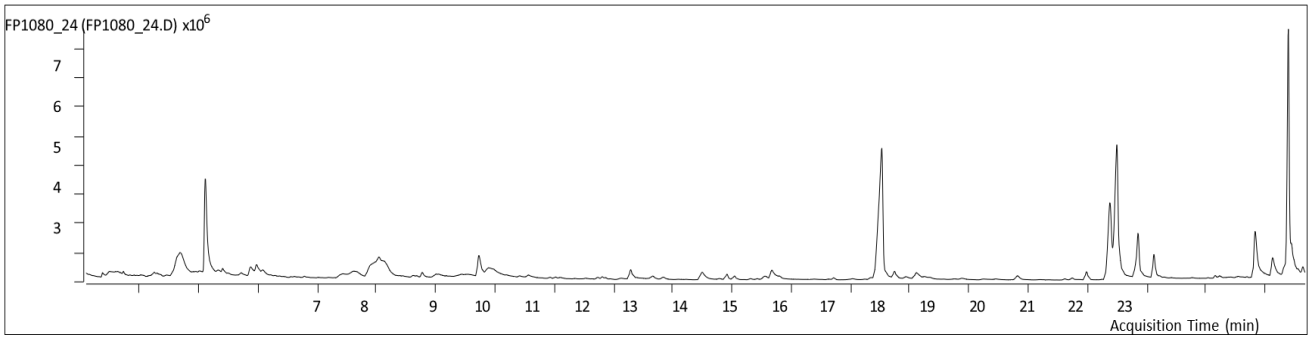

Figure 3: GC-MS Chromatogram of methanolic extract of A. aculeata. The spectrum obtained from the GC-MS analysis is shown.

Table 7: Pharmacological activities of compounds in *A. aculeata*. The table shows compounds found the GC-MS analysis with reported pharmacological activities in literature. Of the 32 compounds that were found in the GC-MS analysis, 17 have reported pharmacological activity in literature.

Name of compound	Reported pharmacological activity	References
Benzofuran, 2,3-dihydro	Antibacterial Antiparasitic Anti-inflammatory	(Hiremathad et al., 2015) (Miao et al., 2019)
Nonanoic acid	Topical antibacterial Fungicide	(Sahin et al., 2006)
Dodecanoic acid	Antibacterial Antifungal Antiviral Anti-inflammatory	(Ameena et al., 2024)
3-Butylisobenzofuran-1(3H)-one	Antibacterial Antifungal Anti-inflammatory Analgesic	(Miao et al., 2019)
2,6-Diisopropylnaphthalene	Pesticidal	(US EPA, 2003)
Tetradecanoic acid	Nematicide Antifungal	(Momodu et al., 2022)
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-	Anti-inflammatory	(Wang et al., 2011)
Neophytadiene	Anti-inflammatory Antimicrobial Antimicrobial Analgesic Antipyretic	(Rajeswaran & Karthick, 2025)
2-Pentadecanone, 6,10,14-trimethyl-	Antibacterial Anti-inflammatory	(Khan & Javaid, 2022)
n-Hexadecanoic acid	Anti-inflammatory Pesticidal Nematicide Antimicrobial	(Mazumder et al., 2020) (Gopu et al., 2021) (Krishnan et al., 2016)
Tetradecanamide	Antibacterial	(Pal et al., 2014)
Phytol	Antimicrobial Immune-modulating Antiparasitic Anti-inflammatory	(Silva et al., 2014) (Taj et al., 2021) (Carvalho et al., 2019)
9,12-Octadecadienoic acid (Z,Z)-	Anti-inflammatory Antibacterial	(Manilal et al., 2009)
cis-Vaccenic acid	Antibacterial Anti-inflammatory	(Yazıcı, 2024) (Semwal et al., 2018) (Jacome-sosa et al., 2016)
Octadecanoic acid	Anti-inflammatory Hepatoprotective effect Antiviral Antibacterial	(Okeke et al., 2024) (Entigu et al., 2013) (Phillips et al., 2015)

Hexadecanamide	Neuroprotective effects Anti-inflammatory Antibacterial	(Bao et al., 2023)
9-Octadecenamide, (Z)-	Anti-inflammatory Antibacterial	(Nonthalee et al., 2023) (Setiawan et al., 2023)

4. Discussion

The yield of extracted chemicals is influenced by the solvent's polarity (Ghasemzadeh et al., 2011; Nawaz et al., 2020). Extraction yield was high when distilled water and ethanol, which are polar solvents, were used. Nawaz et al., (2020) in their studies concluded the same in their results that the extraction yield was high in highly polar solvents. High polar solvents in most cases, give higher yields for extracts and higher extraction efficiencies from plants because a variety of phytochemicals are polar or moderately polar (Diem Do et al., 2013). Distilled water has a polarity index of 9, while ethanol, though being polar, has a lower polarity index of 5.2 according to the Snyder scale (Snyder, 1974) thus justifying higher yields in distilled water extracts than ethanol extracts. Extraction yield was significantly reduced when hexane was used as an extraction solvent. Hexane is a non-polar solvent with a polarity index of 0.1 (Snyder, 1974), which explains the low extraction yield in extracts from the solvent.

Despite variations in yield from the extraction solvents, other factors may have an impact. Methodology, solvent-to-solid ratio, extraction solvent, time, and particle size are different factors that can affect the amount of yield that is extracted (Azwanida, 2015). In the current study, the extraction method used, which was the maceration technique and the extraction time were the same across all solvents. In this regard, the extraction solvents become the sole cause of the variations in extraction yields. A higher yield indicates that more phytochemicals have been extracted, which increases the biocidal activity (Nawaz et al., 2020).

Although the drying process can alter the nutritional, physical, and chemical properties of leaves, it generally leads to a concentration of nutrients. This likely explains why dried Aloe aculeata leaf samples produced higher extraction yields compared to fresh leaves (Youssef & Mokhtar, 2014). Active nutrient levels in dried leaves are usually three to four times greater than in fresh leaves. However, when the solid-to-solvent ratio decreases, the concentration of the extract also declines (Predescu et al., 2016). Due to their high moisture content, fresh *Aloe* leaves, especially the gel, have a lower solid-to-solvent ratio (Sabat et al., 2018).

Phytochemicals can be either polar or non-polar, so the choice of solvent plays a crucial role in dissolving specific plant compounds (Kumarasamy & Senthamarai, 2020). During extraction, the solvent penetrates the plant material and dissolves bioactive compounds that share similar polarity (Nortjie et al., 2022). The extraction of various phytochemicals, including saponins, alkaloids, anthraquinones, terpenoids, and coumarins, was seen to be more effectively done in polar solvents (distilled water and ethanol) than in the non-polar hexane solvent. Polar solvents tend to extract phytochemicals that are polar or have polar functional groups, which include flavonoids, phenolic compounds, tannins, alkaloids, and saponins. Compounds with hydroxyl, carboxyl, or amino groups dissolve better in polar solvents (Sasidharan et al., 2011).

The phytochemical analysis done in the current study has detected a number of phytochemical compounds in *Aloe aculeata*, including alkaloids, flavonoids, tannins, phenolic compounds, and saponins, which are responsible for the medicinal plant's therapeutic properties (Nwozo et al., 2023). The preliminary screening test for phytochemicals is helpful in identifying the bioactive principles, which could then lead to the development and discovery of new drugs. The bioactive components of a plant that have a physiological effect on an animal are what give it its therapeutic value (Rochfort et al., 2008). Plant secondary metabolites are widely used in human disease treatment, veterinary medicine, agriculture, and scientific laboratory research (Elshafie & Camele, 2023).

A wide range of phytochemicals from various chemical classes inhibit different kinds of bacteria *in vitro* (Yadav & Agarwala, 2011). Tannins detected in *Aloe aculeata* leaves have antiviral properties (Vilhelmova-ilieva et al., 2019), demonstrating *Aloe aculeata*'s perceived effectiveness in the management of Newcastle disease, a viral disease in drylands of Masvingo province (Gobvu et al., 2024). Tannins have antibacterial, antiparasitic, anti-inflammatory, and antioxidant properties for potential therapeutic applications (Tong et al., 2022). Plant steroids are recognized to have insecticidal and antibacterial effects (Yerlikaya et al., 2023).

Alkaloids had a concentration of 3.2% and the presence of alkaloids in *A. aculeata* leaves justifies the use of *Aloe aculeata* in poultry health management. Alkaloids exhibit a wide range of biological activities that are of pharmacological value in animal health, including antimicrobial and immunomodulatory, thus helping in improving resistance to infections (Pereira et al., 2023; Riaz et al., 2023). Saponins with a concentration of 3.15% possess an extensive range of pharmacological properties, including anti-inflammatory, immune stimulation, antiparasitic, antifungal, antiviral, and antibacterial (Barbosa, 2014).

The therapeutic potential of phytochemicals in medicinal plants has attracted growing interest globally, as there is an increasing preference for natural and safer treatment options (Ashraf et al., 2023). The antibacterial activities of the phytochemicals detected in *Aloe aculeata* leaves are thus important as the use of the leaves can help inhibit the growth of bacteria such as *E. Coli* helping prevent enteric infections in poultry like Colibacillosis (Patra & Saxena, 2009). Immune stimulation characteristics in phytochemicals from *A. aculeata* leaves can promote the plant's use as an immune booster in poultry by stimulating macrophage activity (Kim et al., 1999) and cytokine production (Yesilada et al., 2005), thereby supporting disease resistance in poultry. Anti-parasitic effects of secondary metabolites assist in the reduction of internal parasite load by disrupting parasite membranes (Wink, 2012).

The observations cited on phytochemical compounds detected in the current study provide scientific support for the ethnoveterinary uses of *Aloe aculeata* leaves as reported in field surveys (Gobvu et al., 2024), thus aligning indigenous knowledge with laboratory evidence. As such, it can be suggested that *A. aculeata* leaves can be used as an antimicrobial, anti-inflammatory, and antiparasitic agent and can be promoted to be used in the future development of poultry drugs.

Identifying functional groups in plant extracts provides insight into their bioactive components (Altemimi et al., 2017). This information is crucial, as it helps determine the compounds responsible for therapeutic effects like antioxidant, antibacterial, and anti-inflammatory activities (Kumar & Pandey, 2015). FT-IR peaks presented the presence of the carbonyl group. The carbonyl group is a functional group in bioactive compounds contributing to their pharmacological activities (Matosiuk et al., 2001). The carbonyl-containing compounds are associated with antibacterial and antifungal activity and can disrupt bacterial and fungal cell walls (Glomb & Swiatek, 2021). The carbonyl group's polarity and electrophilicity makes it reactive and form bonds with nucleophilic sites on biological sites like enzymes, contributing to medicinal activity (Matosiuk et al., 2001).

The hydroxy group is associated with antimicrobial activity as they can interact with microbial cell membranes, leading to inhibition or disruption through forming hydrogen bonds with key amino acids in bacterial enzymes (Konuk & Ergüden, 2020). The aromatic ring functional group is a core structure in many bioactive compounds (Ertl et al., 2020). Aromatic rings contribute to antibacterial activity by binding to microbial enzymes and disrupting cell membranes and also interfering with viral replication (Meyer et al., 2003). Sulfur oxy compounds interfere with folic acid synthesis in microbes thus acting as antimicrobial agents (Fern & Aguilar, 2019). Sulfonated compounds can inhibit viral attachment and fungal cell wall synthesis (Ghidoli et al., 2021; Oza et al., 2024). Primary amino groups are highly important in pharmacology due to their chemical reactivity and ability to form hydrogen bonds, enabling improved binding affinity on the interaction of the herbal medicine and the target (Cozzone, 2002).

The detection of the different functional groups through FT-IR and their supposed pharmacological activities are evidence of the efficacy of *Aloe aculeata* leaves as an ethnoveterinary medicine and its future use for drug development.

Terpenoids detected in *A. aculeata* leaves have various biological properties, amongst which are anti-inflammatory, antiparasitic, which justify the mentioned use of the plant in managing internal parasites in communal areas of Zimbabwe (Gobvu et al.,2023; 2024). Terpenoids have analgesic, antibacterial and antiviral activities (Singh et al., 2023).

Flavonoids, which had the highest concentration (5.6%) in quantitative analysis of *A. aculeata* leaves, indicating their dominance in the phytochemical profile of the extract. The high flavonoid content suggests potential of antibacterial, antiviral, and anti-inflammatory activities (Awang-Kanak et al., 2019) in the *Aloe aculeata* leaves, hence its wide use for variety of poultry diseases in drylands of Masvingo (Gobvu et al., 2024).

GC-MS analysis of the Aloe aculeata leaves revealed a total of 32 compounds, out of which 17 have documented pharmacological activities including antibacterial, antiviral and antiparasitic (**Table 7**), these findings provide scientific support for the traditional use of *Aloe aculeata* as an ethnoveterinary medicine in managing poultry diseases. The high number of compounds with pharmacological activity shows that Aloe aculeata is a plant of great importance in use for development of herbal drugs for the management of poultry health. n-hexadecanoic acid was the most abundant compound in A.

aculeata leaves and has reported anti-inflammatory, pesticidal, nematicidal and antimicrobial activities (Krishnan et al., 2016). 9,12-Octadecadienoic acid (Z,Z)-, n-hexadecanoic acid and phytol which were identified in the current study have been reported to be present in *Aloe vera* (Arunkumar & Muthuselvam, 2009); a species from the same genus as *Aloe aculeata*.

The compounds detected from GC-MS analysis belong to six different chemical classes which include fatty acids for example n-hexadecanoic acid (palmitic acid) and cis-vaccenic acid (Holloway & Wakil, 1964); fatty acid amides for example 9-Octadecanamide (Z)-(oleamide) (Hiley & Hoi, 2007); ketones for example 2-Pentadecanone,6,1014-trimethyl-; alcohols and diterpenes for example phytol (Silva et al., 2014); aromatic and fused ring compounds for example benzofuran,2,3-dihydro- (Wang et al., 2011) and hydrocarbons/ terpenes for example neophytadiene (Gonzalez-rivera et al., 2023).

Fatty acids exhibit antimicrobial activity through bacterial membrane disruption leading to cell lysis, interfering with RNA/DNA replication impeding nucleic acid synthesis thus affecting bacterial proliferation (Fischer et al., 2012). Fatty acids can influence cytokine production by enhancing host defense mechanisms aiding in faster clearance of pathogens like bacteria. Cis-vaccenic acid is a monounsaturated omega-7 fatty acid known for its bacteriostatic properties against some Gram-negative bacteria. Research has shown that it exhibits antimicrobial activity against both reference and clinical strains of Pseudomonas aeruginosa, with effectiveness increasing alongside its concentration (Yazıcı, 2024). This thus justifies the mentioned *Aloe aculeata*'s use in managing bacterial diseases in poultry like diarrhoea. Tetradecanamide, a fatty acid amide, is cited for its gelation characteristics (Pal et al., 2014), the unique characteristic has pharmacological advantages in drug delivery systems where it facilitates enhanced stability and bioactivity. Such gel-forming compounds support formulation herbal based remedies for topical application and wound management in poultry justifying *Aloe aculeata*'s use as an ethnoveterinary medicine.

Phytol, a diterpene alcohol has demonstrated pharmacological activities. Phytol demonstrates strong antimicrobial activity, showing effectiveness against a broad range of bacteria, fungi, and viruses (Taj et al., 2021) making it valuable in disease control in poultry. Aromatic and fused ring compounds play a vital role in pharmacology due to their diverse structural frameworks and biological activities (Bhagwat & Ambre, 2023). Aromatic compounds enhance the binding affinity of bioactive molecules to microbial enzymes and receptors, thus increasing therapeutic potency (White, 2023). Benzofuran-2,3-dihydro- and its derivatives have notable pharmacological activities that include antimicrobial, antiprotozoal and antiparasitic due to the benzofuran scaffold, which is common in biologically active compounds (Hiremathad et al., 2015). The effects are particularly relevant in the context of poultry health, where gastrointestinal infections and coccidiosis are prevalent. Benzofuran scaffolds possess anti-inflammatory and antioxidant activities (Miao et al., 2019) which contribute to improved gut integrity and overall immunity in birds.

The study used a triangulation approach to assess the phytochemical composition of Aloe aculeata leaves, enhancing the reliability and validity of the results (Lagu & Kayanja, 2010). FT-IR analysis confirmed the presence of phenols, which was consistent with findings from the preliminary phytochemical screening. Flavonoids are a class of plant phenols identified through qualitative phytochemical screening. Plant phenols have antioxidant, antibacterial, and anti-inflammatory activity (Zhang et al., 2022). FT-IR analysis detected the presence of amines; alkaloids detected in phytochemical analysis are a class of amines (Matsuura & Fett-neto, 2015), thus indicating agreement of results from the methods. Quinones were detected by both FT-IR and qualitative phytochemical analysis. Quinones demonstrate antibacterial activity against a broad spectrum of bacteria under both *in vitro* and *in vivo* conditions (Mohanty et al., 2022).

Phytol, which was revealed from the GC-MS analysis, is a branched chain unsaturated alcohol (Silva et al., 2014); the FT-IR assay indicated the presence of alcohols. Upon classification of compounds from GC-MS, they revealed some functional group chemical classes that came out in the FT-IR analysis, and these include ketones (carbonyl group), alcohols, fatty acid amides containing amino group(s), and aromatic compounds. As such, the results from the different methods utilised for phytochemical analysis were in agreement and can confirm the reliability and validity of the findings.

5. Conclusion

The bioactive phytochemical substances found in ethnobotanical plants like *Aloe aculeata* have promising pharmacological properties and can be used as medicinal substitutes. This study sought to provide scientific validation for the traditional usage of *Aloe aculeata* leaves by farmers in Zimbabwe's dryland areas to treat poultry diseases. Thirteen of the 32 compounds included in the GC-MS profile have pharmacological activity in published studies. FT-IR analysis of *A. aculeata* leaves established the presence of different functional groups, including the aromatic ring, hydroxy group, carbonyl group,

sulfur oxy compounds, and primary amino group. The qualitative phytochemical analysis revealed that *A. aculeata* contained alkaloids, phenolics, tannins, saponins and flavonoid compounds amongst others. It can be concluded that the presence of different phytochemicals found using different techniques suggests that *Aloe aculeata* may serve as a potent source of medicine. The presence of various phytochemical constituents in *Aloe aculeata* leaves supports farmers' use of the leaves to treat a variety of chicken ailments. In this regard, *A. aculeata* can be taken into consideration as a potential source of phytopharmaceutical value.

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Conflict of interest

The authors declare no conflict of interest

Data availability statement

All data generated during the study are included in this manuscript.

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