

Gc-MS Profiling and in Vitro Anti Coccidial Activity of the Ethanol Extract of Solanum Dasyphyllum Fruit on Broilers

E. James Akpojevbe

edibimi@gmail.com

National Veterinary Research Institute

Fatima Sawa

Abubakar Tafawa Balewa University

Toma Buba

Abubakar Tafawa Balewa University

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GC-MS PROFILING AND IN VITRO ANTI COCCIDIAL ACTIVITY OF THE ETHANOL EXTRACT OF SOLANUM DASYPHYLLUM FRUIT ON BROILERS

ABSTRACT

Coccidiosis is a deadly disease that interferes with the efficiency and well-being of chickens. This disease is caused by a parasite of the genus *Eimeria*, which has seven known species affecting different parts of the intestinal tract of chickens. It is a major menace to the global poultry industry and this has resulted in the need to explore alternative treatment options.

This study aimed to evaluate the anti-coccidial potential of the ethanol crude extracts of *Solanum dasyphyllum* fruit plants used in the treatment of poultry diseases and identify the phyto- constituents using GC-MS technique.

Three different concentrations of the ethanol crude extract of *Solanum dasyphyllum* fruit were used, along with Amprolium (Standard drug) and Water (negative control). 2ml and 10ml of the extract were placed in separate petri dishes labelled A₁-E₁ and A₂-E₂ respectively. Approximately 0.5ml of 6×10^3 suspensions of unsporulated oocysts and sporulated oocysts were dispensed in the separate Petri dishes A₁-E₁ and A₂-E₂. The set up was allowed to stay for 48hrs for unsporulated and 72hrs for sporulated at room temperature, semi-covered with periodic homogenization. 0.1ml suspension of each dilution was examined microscopically after 24 and 48 hours for inhibition of sporulation and 72 hours for damaged oocysts in A₂-E₂. Number of non-sporulated, and deformed oocysts per 100 oocysts were counted respectively. The crude ethanol extract of *S. dasyphyllum* leaves were subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

Results: The different extract concentrations had anti-coccidial activities and there was a significant difference ($P < 0.05$) in the efficacies of the different concentrations (25, 50 and 100mg/ml) compared to the negative control. 100mg/ml exerted the greatest percentage (448) (95.1%), (324) (93.2%) and (330) (92.10%) oocyst lethality. The observed efficacy was concentration dependent. GC-MS analysis showed 6 prominent bioactive compounds, all of which are known to have anti-microbial, anti-oxidant, anti-inflammatory and anti-fungal properties.

This study therefore elucidates the potentials of the *Solanum dasyphyllum* plant as a good source of bioactive compounds and the in-vitro results shows that the extract significantly inhibits oocyst sporulation in a dose –dependent manner.

Keywords: Gas Chromatography-Mass Spectrometry (GC-MS), Oocyst, *Solanum dasyphyllum*, *Eimeria*, Sporulation

Introduction

Medicinal plants have been used throughout human history for the treatment of both human and animal disease conditions (Heinrich *et al.*, (2015).

This has generated renewed interest in recent times, as herbal preparations are increasingly used in health care systems to treat various animal diseases especially in poultry farming. (Xiao *et al.*, 2014; Pashaei *et al.*, 2024)

Phytochemicals derived from these plants are gaining attention as potential alternatives for the treatment of coccidiosis due to the broad spectrum of their biological activities and perceived safety.

Due to the frequency of drugs and vaccine failure, attention has been drawn towards plant-based phytochemicals. Medicinal plants are now used in different countries (Pashaei *et al.*, 2024; Wendimu *et al.*, 2024) The prevention of diseases, enhancement of growth, feed intake and feed efficiency are critical factors in modern animal production today (Gonzalez Ronquillo and Vargas-Bello-Pérez, 2022; Fernández-Miyakawa *et al.*, 2024). Substitution of synthetic drugs with plant-based supplements could ensure healthy food for the human population, reduce the reliance on synthetic drugs and thus reduce the development of pathogen resistance. Bioactive plants and their compounds may assist in this aspect as they are often less expensive, well received by consumers and are generally considered to be environmentally safe (Zhang *et al.*, 2024; Tekeste *et al.*, 2021).

Several plants have been reported to possess anticoccidial activity such as *Khaya senegalensis*, *Azadirachta indica* and *Solanum dasyphyllum* (Gotep *et al.*, 2016; Ibrahim, 2016).

Solanum dasyphyllum is a shrub native to South America, found in countries like Peru, Bolivia, Ecuador, Nigeria and other African countries. It is characterized by its dark green leaves covered in soft hairs, giving them a velvety texture. The leaves are deeply lobed and irregular in shape.

Avian coccidiosis is a common enteric parasitic disease of poultry, and a major constraint to the success of poultry farming in Nigeria. (Lawal *et al.*, 2016).

Protozoan parasite of the genus *Eimeria* with about 1,800 species is reported to colonize and develop within the intestinal tract of animals. (Lawal *et al.*, 2016; Duszynski and Upton, 2022).

Seven species cause infection in chickens, of which six have specific predilection sites for development. *E. acervulina*; duodenum, *E. mitis* and *E. maxima*; middle part of the small intestine etc. (Raman *et al.*, 2011; López-Osorio *et al.*, 2020).

The diversity of *Eimeria* species and their host specificity adds an extra layer of complexity to the problem. Different species may manifest in various ways in different animals, necessitating a multifaceted approach to the disease prevention and control. It is against this backdrop that this study is embarked upon, to assess the chemical composition and *in vitro* effects of *Solanum dasyphyllum* fruit extract on *Eimeria* Species in broilers and also carry out GC-MS analysis to evaluate the various bioactive compounds present.

Materials and methods

Plant Collection and Authentication

The fruits of *Solanum dasyphyllum* were collected in K-Vom, Jos South Local Government Area of Plateau State, Nigeria. The specie was authenticated and recorded by the resident taxonomist at the College of Animal Health and Production Technology, Vom, Plateau State, Nigeria.

Sample Preparation and Extraction

The fruits of *Solanum dasyphyllum* were washed properly and dried at room temperature and pulverized using a pestle and mortar for extraction. The powdered fruit plant (particle size of 0.75mm) was macerated in ethanol in a solid- solvent ratio of 1:4 (1kg in 4L) for 72 hours at room temperature and filtered to obtain the filtrates. Filtrates were completely evaporated using a hot air oven at 45°C to obtain the ethanol extracts of the fruit plant.

Fruit plant extract yields

The yield was calculated for the extract using the following formula after solvent evaporation and stored in bottles at 4°C in the refrigerator till usage.

$$\text{Yield} = \frac{\text{Mass of extract}}{\text{Mass of dried plant powder}} \times 100$$

Phytochemical analysis

Phytochemical analysis of the extract was conducted using qualitative techniques outlined by Builders *et al.* (2011). The metabolites tested for include: phenolic compounds, alkaloids, flavonoids, phenones, coumarine tannins, saponin, triterpenes and steroids.

Preparation of test solutions

A stock solution for the ethanolic fruit extract with a concentration of 100 mg/ml was prepared. 5000 mg of dried extract was weighed using an electronic scale balance and dissolved in 10 ml of distilled water in a mortar and stirred until it had fully dissolved. 40 ml of distilled water was then added and the homogenate was transferred into a beaker. Subsequent concentrations of 50 mg/ml and 25 mg/ml concentrations were produced from the stock solution by serial dilution (dilution factor 2) for oocysticidal evaluation.

Anti-Coccidial Activity Assays

In vitro sporulation inhibition of extract on broiler *Eimeria* oocysts

Clinical oocysts of avian *Eimeria* were obtained from the Parasitology Division of National Veterinary Research Institute, Vom.

2 ml of the different concentrations of the extract (25 mg/ml, 50 mg/ml, and 100 mg/ml), Water (negative control) and 1 mg/ml of Amprolium (positive control) were placed in separate petri dishes. They were labeled A, B, C, D and E respectively. 2ml suspension containing 6×10^3 unsporulated oocysts were dispensed into the petri dishes and incubated at 28°C. The sporulated and unsporulated oocysts were counted using a McMaster chamber counter.

The set up was examined after 24 and 48 hours under a light microscope using objective 40x and repeated three times for each treatment and control in the same

conditions. The number of sporulated oocysts and non- sporulated oocysts were counted and the percentage of sporulation was estimated by counting the number of sporulated oocysts in a total of 100 oocysts. The sporulation inhibitory percentage was calculated as defined by (Abbas *et. al.*, 2012).

$$(\text{Sp})\text{IP} (\%) = \frac{\text{Sp \% of control} - \text{Sp \% of extract}}{\text{Sp \% of control}} \times 100$$

Where: (Sp) IP = Sporulation inhibition percentage

Sp = Sporulation

In vitro viability inhibition of extract on the sporozoites of broiler Eimeria

10mls of the three different concentrations of the extract (25mg/ml, 50mg/ml, 100mg/ml, Water (negative control) and 1mg/ml of Amprolium) were placed in separate petri dishes. These were labeled A, B, C, D and E respectively. To each petri dish, 0.5 ml suspension containing 6×10^3 sporulated oocysts was added. These were kept at room temperature, semi-covered with periodic homogenization. 0.1 ml of each concentration was examined microscopically after 72hrs. The experiment was repeated three times for each concentration and control treatment under identical settings. The number of viable and nonviable (Damaged) sporozoites was calculated, and the percentage of viability was estimated by counting the number of viable sporozoites in a total of 100 sporozoites using McMaster chamber counter. The percentage inhibition of viability was calculated as defined by (You, 2014).

$$\text{Viability inhibitory (Vi)} = \frac{\text{Vi\% of control} - \text{Vi\% of extract}}{\text{Vi\% of control}} \times 100$$

GC-MS Analysis

The ethanol extract of *S.dasyphyllum* was screened for bioactive compounds by Gas Chromatography-Mass Spectrometry (GC-MS). The standard method according to Oyinloye *et al.* (2023) was adopted.

Statistical analysis

Spss software was used for the statistical analysis

Data obtained from in-vitro anti-coccidial tests were subjected to two-way analysis of variance (ANOVA). Least Significant Difference (LSD) was used to separate the means where significant difference occurs among the efficacies of the different concentrations from the plant extract.

Results

Percentage yield and Phytochemical Screening

The extract yield was calculated to be 50.3g from the total weight of 1000g of the pulverized *Solanum dasyphyllum* fruit material and the phytochemical constituents were analyzed (Table 1). Table 2 shows the phytochemical result of the ethanol fruit extract of *Solanum dasyphyllum* when tested for the presence of phenolic compounds, alkaloids, flavonoids, tannins, saponin, triterpenes, steroids and polyphenols.

In vitro anti-coccidial/oocysticidal activities of the extract

Results presented in Table 3 showed in vitro oocysticidal sporulation inhibition activities against broiler *Eimeria* oocysts ($p < 0.05$) at different concentrations from ethanol extract of *S. dasyphyllum* fruit at 24hr and 48hr compared to negative control. The results showed that the fruit plant had significant effects on the sporulation of broiler *Eimeria* oocysts. The number of sporulated oocysts decreased with increasing extract concentrations. The highest efficacy after 24hr and 48hr of incubation was $64.33 \pm 9.2\%$ and $100 \pm 0\%$ respectively at the concentration of 100mg/ml of the ethanol fruit extract against broiler *Eimeria* oocysts. The lowest efficacy was $41.67 \pm 8.0\%$ and $90.33 \pm 3.7\%$ at the concentration of 25mg/ml after 24hr and 48hr of incubation. The Table 3 also shows the influence of incubation time on the sporulation of *Eimeria* oocysts and it is observed that the inhibition rate increased with increased incubation time. The highest efficacy of the fruit extract was recorded at 48hr post inoculation. The results confirm the dose-dependent efficacy of *S. dasyphyllum* fruit extract in inhibiting *Eimeria* sporulation. At 25 mg/ml, the extract achieved 90.33% inhibition, while higher concentrations (50 mg/ml and 100 mg/ml) achieved complete inhibition (100%), comparable to the positive control.

Table 4 showed the viability inhibition of the extract on broiler *Eimeria* sporozoites at different concentration ($p < 0.05$) compared to the negative control. The results showed that the fruit extract had significant effect on the viability of the *Eimeria* sporozoites at the different concentrations of (25, 50 and 100mg/ml). The number of damaged sporozoites increased with increasing fruit extract concentration slightly. The highest efficacy after 72hr of incubation was 95.07 ± 1.69 at the concentration of 100mg/ml while the lowest efficacy was 92.10 ± 0.92 at the concentration of 25mg/ml of the ethanol fruit extract. There was no significant difference between 25 and 50mg/ml, and 100mg/ml of the extract concentrations at the end of 72hr. The extract at 100mg/ml concentration had no significant difference when compared with the positive control. The result showed that the viability inhibition of the extract is not concentration dependent.

GC-MS analysis of the extract

The result of the GC-MS analysis of the ethanol extract of *Solanum dasyphyllum* revealed varying families of bioactive compounds as shown in Table 5 and the subsequent mass spectra of the detected compounds in Figures 1 (a – g).

Table 1. Yield of the extraction of ethanol extracts of *Solanum dasyphyllum* fruit

Extract	Weight	% Yield
Fruit of <i>Solanum</i> Spp.	1000	53.5

Table 2. Phytochemical screening on ethanolic *Solanum dasyphyllum* fruit extract

Phyto-chemicals	Qualitative data
Saponins	+
Tannins	+
Flavonoids	+

Alkaloids	+
Quinones	+
Coumarin	+
Phenones	+
Steroid/Triterpenoid	+

KEY: (+) detected, (-) not detected

Table 3. In vitro Sporulation inhibition percentage of *Solanum dasyphyllum* fruit extracts on broiler *Eimeria* oocysts

Time	Control (-ve)	Control (+ve)	25	50	100
24hr	7.33±4.00 ^a	80.00±2.6 ^d	41.67±8.0 ^b	51.00±7.6 ^{bc}	64.33±9.2 ^{cd}
48hr	0.00±0 ^a	100±0 ^c	90.33±3.7 ^b	100±0 ^c	100±0 ^c

The results are the mean ± SD of triplicate tests evaluated after 24hr and 48hr of incubation at room temperature. Values carrying different superscript letters are significantly different at $p < 0.05$

Table 4. In vitro Viability inhibition percentage of the ethanol extract of *Solanum dasyphyllum* fruit on broiler *Eimeria* oocysts (% Deformed Oocysts)

Time	Control (-ve)	Control (+ve)	25	50	100
72hr	3.57±1.12 ^a	96.63±0.76 ^c	92.10±0.92 ^b	93.27±1.15 ^b	95.07±1.69 ^{bc}

The results are the mean ± SD of triplicate tests evaluated after 72hr of incubation at room temperature. Values carrying different superscript letters are significantly different at $p < 0.05$

Table 5: Compounds detected in the *S. dasyphyllum* extract

S/N	Retent ion Time	% Peak Area	Compound	Ref	CAS	Similari ty Index
1	17.339	0.82	Acetylacetone, monoxime	7889	100029 8-65-7	91
2	19.390	4.90	1,5-Heptadien-3-yne	2493	003511- 27-1	91
3	20.676	18.40	Pentadecanoic acid, 14-methyl-, methyl ester	13084 1	005129- 60-2	96
4	22.708	12.42	Pentanoic acid	4317	000109- 52-4	91
5	23.965	37.30	9-Octadecenoic acid (Z)-, methyl ester	15575 1	000112- 62-9	90
6	25.881	14.02	Cyclopentane, 1-methyl-2-(2-propenyl)-, trans-	10930	050746- 53-7	90

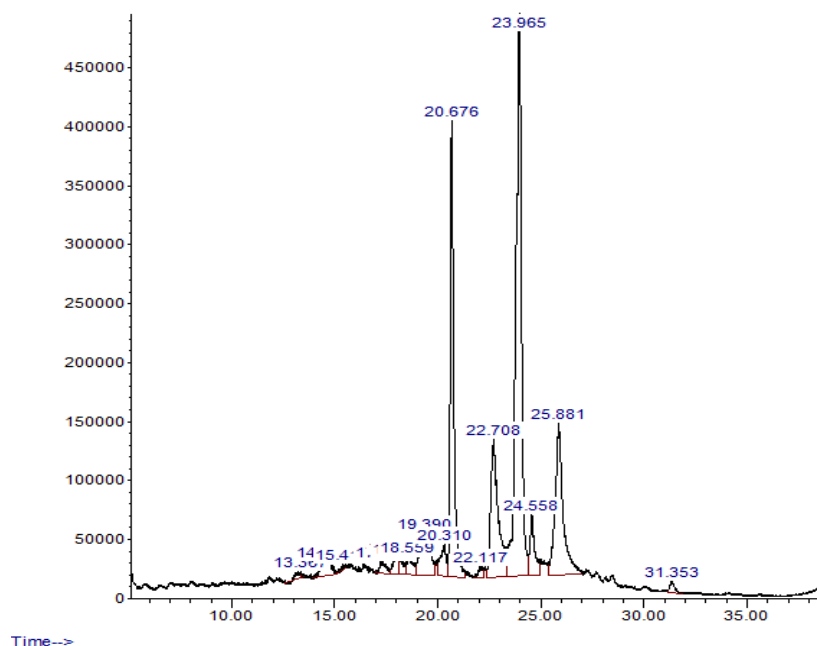


Fig. 1a: GC-MS chromatogram for the whole *S. dasyphullum* extract

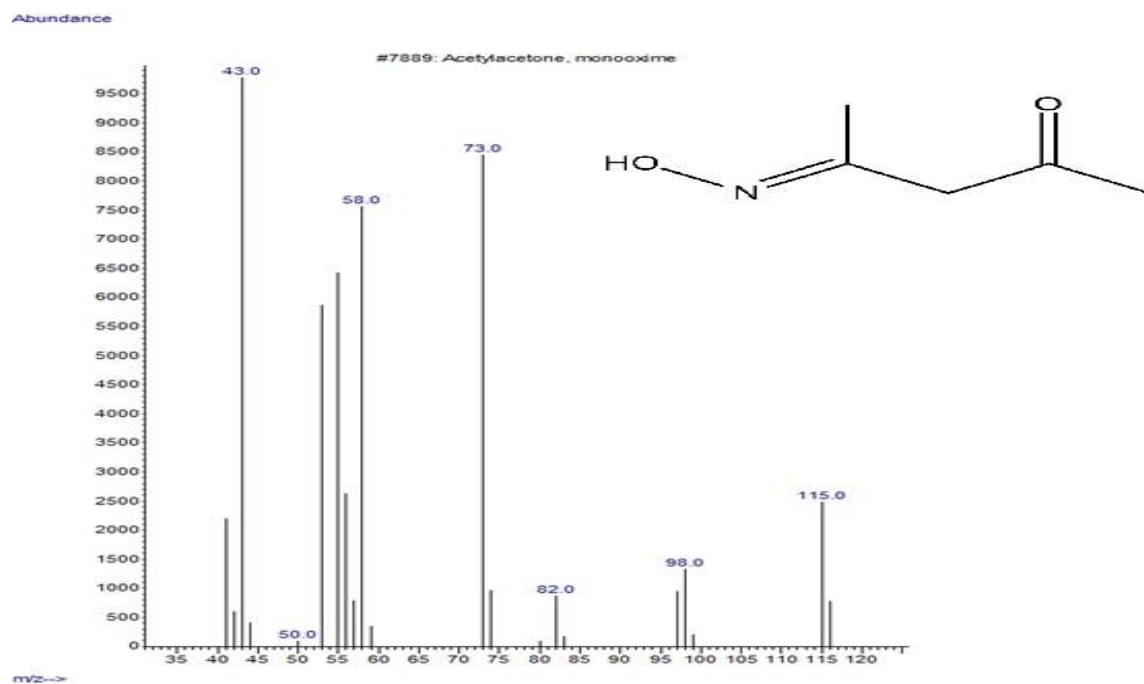


Fig. 1b: GC-MS Chromatogram for Acetylacetone, monooxime

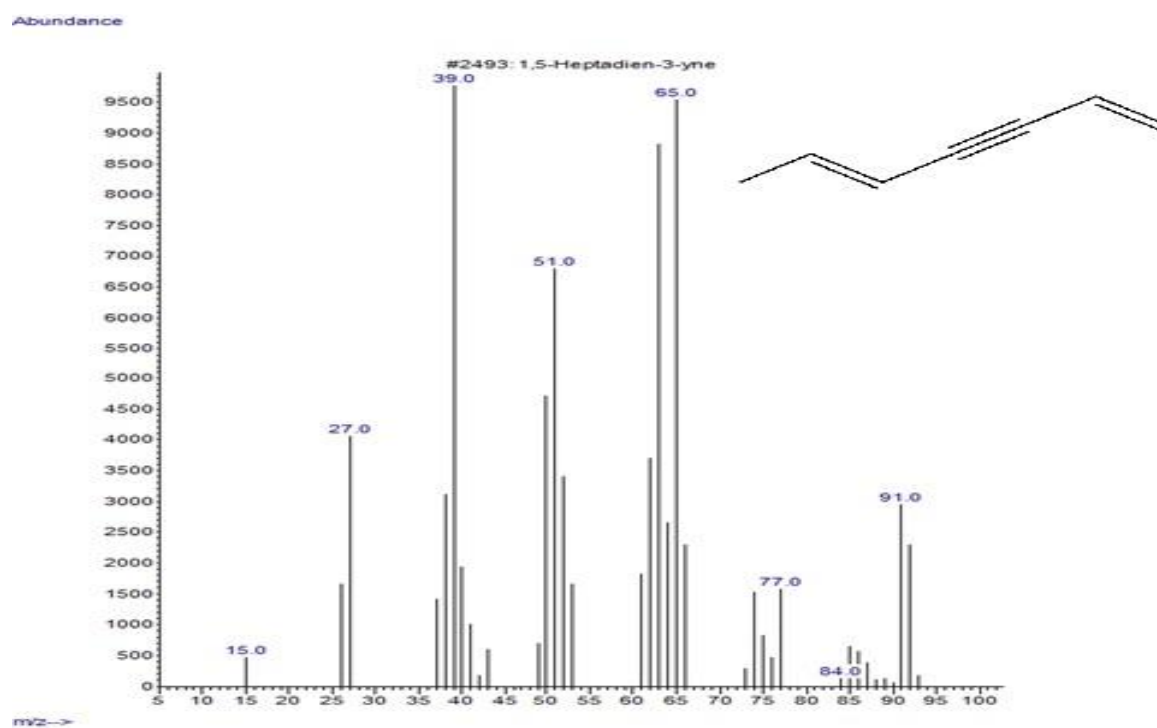


Fig. 1c: GC-MS chromatogram for 1, 5- heptadiene-3-yne

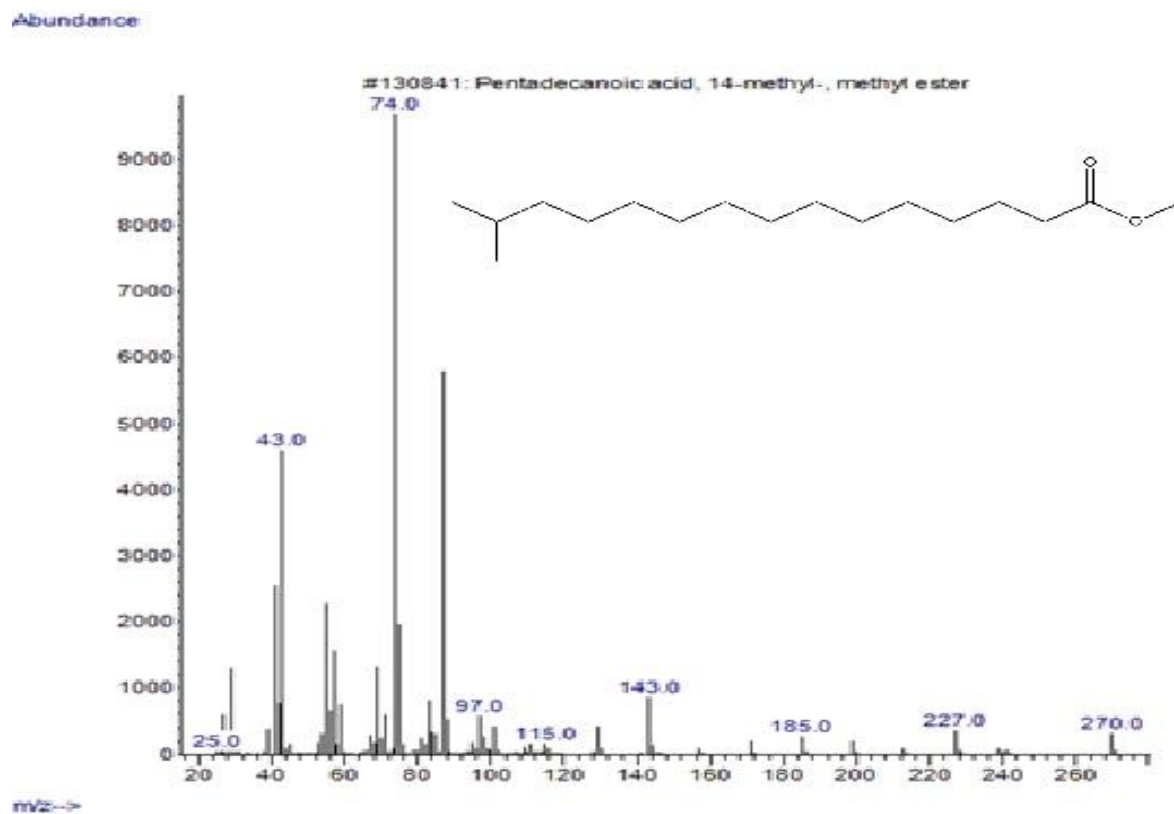


Fig. 1d: GC-MS chromatogram for pentadecanoic acid, 14-methyl ester

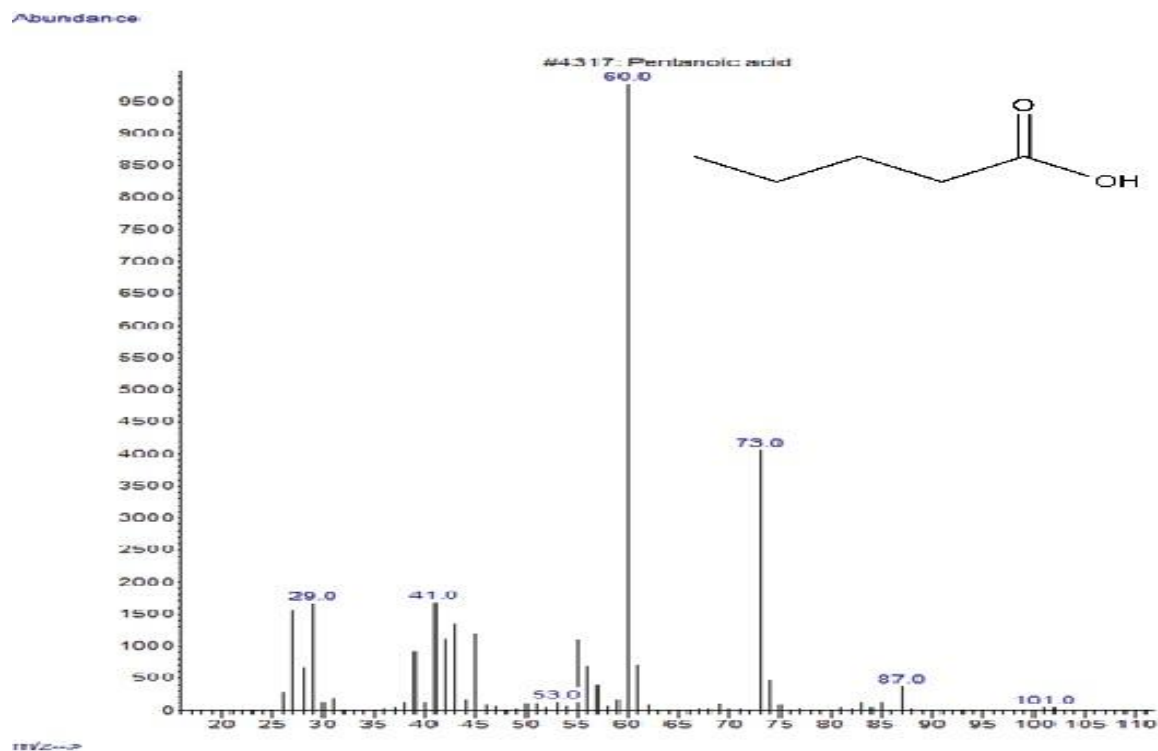


Fig. 1e: GC-MS chromatogram for pentanoic acid

Abundance

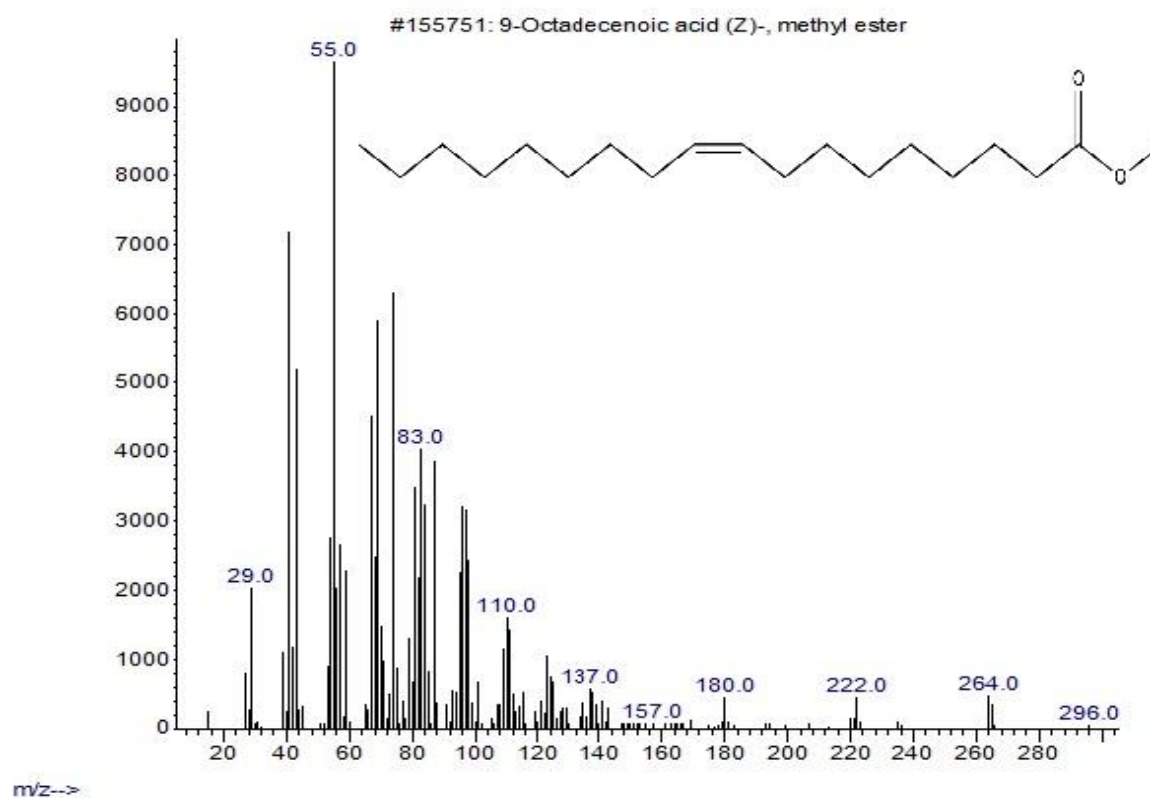


Fig. 1f: GC-MS chromatogram for 9-Octadecenoic acid (Z)-, methyl ester

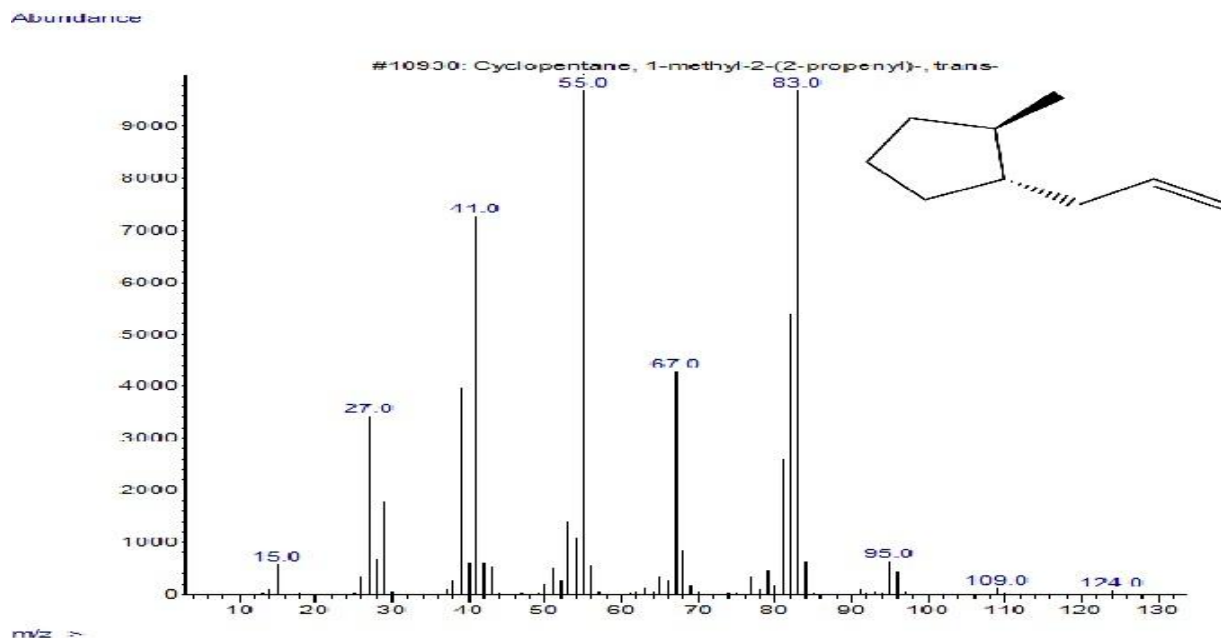


Fig. 1g: GC-MS chromatogram for cyclopentane, 1-methyl-2-(2-propenyl)-, trans-

Table 6. Compounds detected with their biological/medicinal activity in ethanol fruit extract of *S. dasyphyllum*

S/N	Compounds	Molecular formula	Molecular weight (g/mol)	Family of compounds	Medicinal/Biological activity
1	Acetylacetone, monooxime	C ₅ H ₉ NO ₂	115.13	Keto-oxime	Antioxidant, antibacterial and antifungal activities (Khojasteh and Martin, 2015)
2	1,5-Heptadien-3-yne	C ₇ H ₈	92.14	Enynes	No report
3	Pentadecanoic acid, 14-methyl-, methyl ester	C ₁₇ H ₃₄ O ₂	270.45	Fatty acid methyl esters	antibacterial and antifungal activities (Saikarthik et al., 2017)
4	Pentanoic acid	C ₅ H ₁₀ O ₂	102.13	Fatty acid	Flavor component in food; lubricant, used in the manufacture of pharmaceuticals, cosmetic and soap; surfactant and softening agents (Enas & Duha, 2014)
5	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296.49	Fatty acid methyl ester	Antimicrobial, antioxidant, anticancer anemiagenic and anti-androgenic activities (Elaiyaraja <i>et al.</i> , 2018)
6	Cyclopentane, 1-methyl-2-(2-propenyl)-, trans-	C ₉ H ₁₆	124.22	Alkane	antibacterial and antifungal activities (Tice et al., 2017)

Discussion

This study investigated the in vitro anti-coccidial effects of *Solanum dasyphyllum* fruit extract on *Eimeria* oocysts found in broiler birds. The phytochemical analysis of the ethanol extract revealed the presence of several bioactive compounds including saponins, tannins, flavonoids, alkaloids, quinones, coumarins, phenones, and steroids/triterpenoids. The analysis in this study agreed with the research of Ibrahim *et al.*, (2016); Otor *et al.*, (2021) who reported that *Solanum dasyphyllum* fruit contains saponin, alkaloid, tannin, terpenoid, steroid and flavonoid as part of the plant's phytochemical constituents. These compounds are known for their various pharmacological activities, such as antioxidant, antimicrobial, and anti-parasitic effects. The presence of these bioactive components underscores the potential therapeutic value of the extract in combating *Eimeria* species infections. These compounds are known to exhibit antimicrobial, antioxidant, and anti-inflammatory properties, which are vital in addressing parasitic infections like coccidiosis. In comparison, similar studies on *S. dasyphyllum* have identified flavonoids and alkaloids as prominent bioactive components. Eletta *et al.*, (2017) and Sofowora (1993) highlighted their role in providing anti-inflammatory and antiparasitic effects, supporting the results of this study.

Additionally, the results demonstrated that *S. dasyphyllum* extract significantly inhibited oocyst sporulation and sporozoite viability in a concentration-dependent manner. At 100 mg/ml, the extract achieved similar efficacy to Amprolium (1 mg/ml), with minimal sporulation observed after 48 hours. Furthermore, the extract induced substantial deformation in sporozoites, reducing their viability, particularly at higher concentrations. These results highlight the extract's potential as an anticoccidial agent, which could provide a natural alternative to synthetic drugs. The findings are consistent with prior studies, where flavonoids and saponins in plant extracts demonstrated the ability to disrupt the life cycle of parasites. Ajayi (2014) and Cushnie and Lamb (2005) reported that flavonoid-rich extracts interfered with parasitic cell walls, inhibiting sporulation and promoting sporozoite death. Similarly, studies by Eletta *et al.*, (2017) on *Solanum* species confirmed their anticoccidial activity through oxidative stress induction in parasites. However, the degree of efficacy observed in this study exceeds some previous findings, likely due to the optimized ethanol extraction method, which enhances the bioavailability of active compounds (Famuwagun *et al.*, 2017; Xu *et al.*, 1996).

The GC-MS analysis provided a more detailed profile by identifying specific bioactive compounds present in the extract. The major constituents identified were Acetylacetone, monoxime, 1, 5-Heptadien-3-yne, Pentadecanoic acid, 14-methyl-, methyl ester, Pentanoic acid, 9-Octadecenoic acid (Z)-, methyl ester, and

Cyclopentane, 1-methyl-2-(2-propenyl)-, trans-. Among these, 9-Octadecenoic acid (Z)-, methyl ester, exhibited the highest concentration, indicating its possible significant role in the extract's bioactivity. 9-Octadecenoic acid has been associated with anti-inflammatory and anti-parasitic properties (Rasoanaivo *et al.*, 2011), thus its possible relevance in managing *Eimeria* infections. The compound is also reported to have cell membrane-stabilizing and anti-inflammatory effects which are crucial for combating parasitic diseases like coccidiosis (Oyinloye *et al.*, 2021).

The spectra provide molecular ion peaks and fragmentation patterns unique to each compound. Each peak on the chromatogram corresponds to a compound identified based on its retention time and peak area. The retention time indicates the time a compound takes to elute through the chromatography column, while the peak area reflects its relative abundance in the extract. A total of six major compounds were identified, with their retention times ranging from 17.339 to 25.881 minutes.

Acetylacetone, Monooxime was detected with a retention time of 17.339 minutes, making up 0.82 % of the extract, the least abundant compound. Despite its low abundance, it holds significant potential due to its antioxidant, antibacterial, and antifungal properties (Khojasteh and Martin 2015). These attributes suggest its role in mitigating oxidative stress and controlling microbial infections.

1,5-Heptadien-3-yne was present at 4.90% abundance with a retention time of 19.390 minutes. Although its specific biological activity remains under-researched.

Pentadecanoic Acid, 14-Methyl-, Methyl Ester was more prominent, comprising 18.40 % of the extract with a retention time of 20.676 minutes. Known for its antibacterial and antifungal effects, as reported by Saikarthik *et al.*, (2017), this compound significantly contributes to the antimicrobial capabilities of the extract.

Pentanoic acid, with a retention time of 22.708 minutes and making up 12.42 % of the extract, is a versatile compound widely used as a flavouring agent in food and as a surfactant or lubricant in pharmaceuticals and cosmetics (Enas and Duha, 2014). Its industrial applications include roles in stabilization and softening processes.

The most abundant compound, 9-Octadecenoic Acid (Z)-, Methyl Ester, constituted 37.30% of the extract and had a retention time of 23.965 minutes. This compound is a powerhouse of biological activity, displaying antimicrobial, antioxidant, anticancer, and anti-androgenic properties, as noted by Elaiyaraja *et al.*, (2018). Its high abundance underscores its potential as a major contributor to the extract's therapeutic effects, particularly its anticoccidial properties.

Cyclopentane, 1-Methyl-2-(2-Propenyl)-, Trans- was identified with a retention time of 25.881 minutes, accounting for 14.02% of the extract. Its antibacterial and

antifungal properties, highlighted by Tice *et al.*, (2017), add to the overall antimicrobial efficacy of the extract.

The diverse compounds detected in the extract collectively contribute to its diverse therapeutic potential, with many compounds directly linked to antimicrobial and antioxidant activities. This comprehensive profile supports the use of *S. dasyphyllum* as a natural therapeutic agent.

Conclusion

This study proves that the ethanol extract of *Solanum dasyphyllum* fruit possesses significant anti- coccidial activity against broiler Eimeria oocysts. The results suggest that this extract can effectively decrease the number of sporulated oocysts with increased concentrations and efficacy compared to the standard drug, Amprolium. The GC-MS analysis of *S. dasyphyllum* fruit extract confirmed the presence of 6 prominent bioactive compounds, many of which have been associated with antimicrobial, antioxidant, and anti-inflammatory activities. These findings highlight their potential in the treatment of coccidiosis in poultry farming.

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