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In vitro anti-helminthic, antimicrobial, and antioxidant activity of methanolic extract of *Aspilia silphioides* leaves

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Abstract
The leaves of *Aspilia silphioides* have been used traditionally for wound healing, stomach aches, headaches, worm infestations, and birth control. The purpose of this work was to evaluate the antimicrobial, anti-helminthic, and antioxidant activities of the methanolic extract *A. silphioides* and identify the phytochemical components of the extract. Standard methods were employed for the phytochemical screening. The broth dilution method was utilized in assessing the antimicrobial activity. DPPH radical and hydrogen peroxide scavenging assays were employed to assess the antioxidant capacity. *In vitro*, anti-helminthic activity was determined using *Eudrilus eugeniae*. Phenolics, tannins, flavonoids, saponins, glycosides, carotenoids, steroids, polyphenols, phytosterol, quinones, alkaloids, and terpenoids were present in the extracts. As the highest activity of the methanolic extract against *Streptococcus aureus*, a minimum inhibitory concentration of 12.5 mg mL was obtained. The IC_{50} of the methanol extract and the reference drug with regards to the DPPH scavenging activity were 4.66 µg/mL, and 40.29 µg/mL respectively. At 20 µg/mL, the paralysis and death time for both the methanol extract and Mebendazole drug were 25 min, 28 min, 67 min, and 94 min respectively. The IC_{50} obtained for the hydrogen peroxide scavenging assay for the methanol extract and gallic acid were respectively 39.70 µg/mL, and 32.62 µg/mL. Compounds identified in the extract of *A. silphioides* included 9-Octadecenamide, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl) propanoic acid, 17-acetoxy-3a-methoxy-4,4-dimethyl-14-seco-3,9-epoxyandrostane-8,14dione, α-Amyrin, 4-[(2,4-dinitrophenyl) azo]-1-methylethylesterbenzenpropanoic acid, 5aPregn-16-en-20-one-3a,12a-dihydroxy-5a–diacetate. The methanol extract of *Aspilia* silphioides possesses antioxidant, anti-helminthic, and antimicrobial properties as a result of the important phytochemicals it contains.

Keywords: *Aspilia silphioides*, anti-helminthic, phytochemical composition, antimicrobial properties, and antioxidant activity

1. Introduction
Humans depend directly on plants and their products for food, shelter, medicine, clothing, and maintenance of the ecosystem. Thus, the effective use of plants with available technology and knowledge will lead to the survival of humanity (Abbiw, 1990) [1]. Throughout the world, the demand for medicinal plants is increasing due to availability, fewer side effects, and sometimes being the only source of remedy (Lucas, 2010) [22]. Traditional medicine has been used for centuries to cure, treat, prevent, and diagnose diseases (Rai, *et al*., 2016) [37]. Medicinal plants were the main source of medicine in the treatment of various diseases before the advent of Western medicine (Pan *et al*., 2017) [34].
In many developing countries especially in Africa, about 80% of the rural population depends on traditional medicines for primary health care (Appiah *et al*., 2019) [12]. There is a lot of research currently underway, and this is geared toward identifying active compounds in herbal medicine for disease treatment, drug development, and herbal preparation (Newman *et al*., 2007; Asase A., 2011) [28, 13]. In addition, some medicinal plants have been reported to have pharmaceutical properties that make them highly effective in treating several diseases (Abel and Busia., 2005) [2].
Medicinal plants synthesize many chemicals. The phytochemical content and pharmacological effects of some medicinal plants have not been validated by scientific studies to determine their efficacy and safety. However, the antioxidant, antimicrobial, and anti-inflammatory properties of numerous plants have been studied (Acheampong *et al*., 2018; Akoto *et al*., 2019) [3, 8].

Medicinal plant research has paved the way for the discovery of important drugs including digoxin from *Digitalis purpurea* L., morphine from *Papaver somniferum* L., vincristine and vinblastine from *Catharanthus roseus* L., taxol from *Taxus brevifolia* Nut., and others that are used for the treatment of various diseases (Balandrin *et al*., 1993) [14]. Other drugs also derived from medicinal plants are antiparasitic molecules such as quinine and artemisinin and their derivatives (Balandrin *et al*., 1993) [14] which are used alone or in combination with other compounds, in the treatment of malaria. Artemisinin is a sesquiterpene lactone isolated from the Chinese herb *Artemisia annua* (Asteraceae) used to treat fever associated with malaria (Cheuka *et al*., 2016) [17].

Helminth infections pose serious problems to animals and humans, and it is one of the diseases common in subtropical and tropical areas such as the Americas, East Asia, sub-Saharan Africa, and China. Disorders such as malnutrition, anemia, diarrhea, pneumonia, eosinophilia, organ damage, and death in severe cases are caused by parasitic worm infections (WHO 2022; Akoto *et al*., 2019) [49, 19]. Over the years, anthelmintics have been used to minimize losses caused by helminth infections, but resistance to existing anti-helminthics is rife, dating back to 1957 (Van Wyk *et al*, 1999) [47]. Little attention has been given to drug discovery efforts for human helminthiasis (Pena-Espinosa, 2018) [36], hence the listing of some of them (Dracunculiasis, schistosomiasis, onchocerciasis, and soil-transmitted helminths) under the neglected tropical diseases (NTDs) (WHO, 2022) [49]. There is, therefore, the need to screen natural products for anti-helminthic activities that may lead to the discovery of novel anti-helminthic agents.

Biochemical reactions such as cell metabolism and oxidative stress produce free radicals (superoxide, hydroxyl, peroxynitrile, peroxide, nitric oxide radicals), reactive oxygen and nitrogen species (RONS) (Moharram and Youssef, 2014) [25]. Age-related conditions such as neurodegenerative diseases, frailty, cardiovascular diseases, chronic kidney disease, and chronic obstructive coronary disease are caused by the production of these oxidants in the body (Ligouri *et al*., 2018) [21]. Antioxidants reduce the production of oxidants by nullifying pro-oxidant molecules, thereby modulating the oxidant-antioxidant profile of the body system (Ajith *et al*., 2017) [6]. Natural antioxidants have been favored, as they are deemed to have minimal side effects compared with their synthetic analogs, and are cheaper and readily available in many plants (Nasri *et al*., 2013) [27], hence the need to discover more antioxidant molecules from plant sources.

Substances employed to kill or prevent the growth of microbes are referred to as antimicrobials, and the treatment of microbial infections is a big challenge to the world due to the development of multi-drug resistance by the microbes to the drugs used in destroying them. Various antimicrobials have been employed in managing microbial infections including penicillins, tetracyclines, and others. These medicines have a plethora of unpleasant side effects, and microbes have developed resistance to some of them (Anibijuwon and Udeze, 2009) [11]. Research has, therefore, been ongoing over the years to identify and develop new antimicrobials from plant sources (Acheampong *et al*., 2018; Akoto *et al*., 2019; Akoto *et al*., 2020) [3, 19, 7, 9] that may have better efficacy and fewer side effects to augment the existing ones.

A. silphioides is a flowering plant in the family Asteraceae. It is locally known as ‘‘mfofo sika’’. Some species in the genus of Aspilia, such as *Aspilia Africana*, is used in the treatment of diseases such as tuberculosis, cough, measles, diabetes, malaria, and wounds (Okello *et al*., 2021; Okello, Lee, J., & Kang, Y., 2020) [30-31]. Traditionally, *A. silphioides* is used by the Akan people of Ghana and is effective for wound healing, stomach aches, severe pains, blood clots, headaches, worm infestation, and birth control.

The purpose of this study was to determine the pharmacological activities of the methanolic extract of *A. silphioides* including antimicrobial, antioxidant, and anti-helminthic activities, and identify the phytochemical constituents present in the extract.

2. Materials and Methods

2.1 Chemicals and reagents

With the exception of the standard drugs, all chemicals were acquired from Sigma Aldrich Co. Ltd, Irvine, UK. The analytical-grade organic solvents were purchased from BDH Laboratory Supplies in England.

2.2 Sample collection and identification

Samples of *A. Silphioides* were obtained from Bekwai in the Bekwai Municipality of Ashanti region (situated between 25° 43' and 26° 53' North, latitude, and 90° 39' and 92° 11' East latitude) in December 2021. It was authenticated in the Department of Herbal Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. A voucher specimen (KNUST/HM1/2021/L026) has been deposited in the herbarium for future reference.

The leaves were dried under shade at ambient temperature for one month, powdered, and utilized for extraction. The extraction of constituent materials from plants was carried out by cold maceration of the plant sample in methanol (99.9%) at ambient temperature. Known amounts of pulverized sample (100 g) were dissolved in 700 mL of methanol for 72 hours. The resulting extract was filtered, and the filtrate was concentrated using a rotary evaporator under reduced pressure. After the extract had been dried and weighed, the percentage yield of the extract in relation to the pulverized samples was computed. The extract was then kept in a refrigerator at 4 °C until it was required for use in subsequent experiments.

2.3 Qualitative phytochemical analysis

A. Silphioides methanolic leaf extract was screened for the existence of secondary metabolites such as carotenoids, alkaloids, flavonoids, saponins, steroids, cardiac glycosides, phenolics, tannins, polyphenols, and phytosterol, employing the methods described by Trease and Evans (2009) [45].

2.4 *In vitro* anti-helminthic analysis

2.4.1 Worm Collection and Authentication

Behind the Department of Theoretical and Applied Biology at KNUST in Kumasi, Ghana (latitude 6º35–6º40 N and longitude 1º30–1º35 W), the earthworms were gathered from a wet area. The worms were put into a glass container along with some of the dirt they had been retrieved from. Mr. Lawrence Yeboah verified the worm type at the KNUST Zoology Unit of the Department of Theoretical and Applied Biology.

2.4.2 Anti-helminthic Assay

The anti-helminthic assay was carried out using a modified method by Ajaiyeoba *et al*. (2001) [52] as described in our previous article (Osei Akoto *et al*, 2020) [7, 9], and the method description partly reproduces the wording. Stock solution of

the extract at a concentration of 150 g/mL was prepared using distilled water as the solvent. Five additional concentrations of 1.250, 2.50, 5, 10, and 20 g/mL were prepared from the stock solution. Mebendazole was used as the reference standard and sterile distilled water served as negative control. At the beginning of each experiment, fresh test and standard drug solutions were prepared. Into separate Petri dishes containing 50 mL of the various test solutions, four worms of roughly the same size were released. Worms' paralysis and death times were recorded. When the worms did not move other than when vigorously shaken, it was considered paralyzed, and the time was noted as paralysis time. After determining that the worms did not move when vigorously shaken or dipped in warm water (50 °C), and that their body colors had faded, the worms were considered dead and the time it took for them to die was recorded (Ajaiyeoba *et al*., 2001) [52]. The experiment was carried out in triplicate, and the results were presented as mean ± standard error of the mean (SEM).

2.5. *In vitro* Antioxidant activity

Utilizing 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals scavenging and hydrogen peroxide assays, the antioxidant activity was evaluated.

2.5.1 Hydrogen Peroxide Scavenging Assay (H_2O_2)

A modification of the standard procedures by Mukhopadhyay *et al*. (2016) [26] and described in our previous work (Osei Akoto *et al*., 2020) [7, 9] was used to determine the extract's potential to scavenge hydrogen peroxide, and the method description partly reproduces the wording. The specific complexation of 1, 10-phenanthroline with ferrous ion to form a red-orange triphenanthroline complex is the foundation of the assay. A stock solution of Gallic acid of concentration 1000 µg/mL was produced, from which varying concentrations between 200 and 800 µg/mL were obtained by dilution. 0.5 mL of ferrous ammonium sulfate (1 mM) was added to a series of test tubes, and 3 mL of test solutions with varying concentrations of gallic acid solutions or extract were added and mixed. Afterward, 0.13 milliliters of 5 mM hydrogen peroxide was added, then the mixture was incubated for five minutes in the dark at room temperature because hydrogen peroxide can be photobleached. After this, each tube was incubated at room temperature for ten minutes after 3 mL of 1 mM 1, 10-phenanthroline had been added to the mixture. Finally, an ultraviolet-vis spectrophotometer was used to measure the absorbance of the solution at 510 nm. A mixture of ferrous ammonium sulfate (0.5 mL, 1 mM), distilled water (3 mL), and 1,10-phenanthroline (3 mL, 1 mM) formed the negative control. Using the formula below, the percentage of scavenging activity was determined.

$$\% \, Scavenging = [\frac{\text{Atest}}{\text{Acontrol}} \text{ x } 100]$$

Where

$A_{control}$ is the absorbance of the negative control and A_{test} is the absorbance of the test samples. The outcomes were additionally reported in IC_{50} (Mukhopadhyay *et al*., 2016) [26].

2.5.2 1,1 Diphenyl-1-picrylhydrazy (DPPH) free radical scavenging assay

The DPPH radical scavenging assay, as described by Mahdi-Pour *et al*., (2012) [23], was used to measure the antioxidant activity of the plant extract with minor adjustments, and the method description partly reproduces their wording. A universal bottle was used to measure the radical scavenging properties quantitatively. In sterile distilled water, stock solutions of the extract (200 µg/mL) were diluted to concentrations of 20, 40, 60, 80, and 100 µg/mL. A stock solution of concentration 200 µg/mL of ascorbic acid was prepared for the standard, and dilute solutions of concentrations 12.5, 25, 50, and 100 µg/mL were made from it. By adding 50 µL of various concentrations of the test solutions to 150 µL of 0.02 mg/mL 1,1 Diphenyl-1-picrylhydrazy solution, the 200 µL reaction mixtures were produced. The mixtures were then allowed to incubate for 30 minutes at room temperature and in the dark. The absorbance of the mixture was then assessed using a spectrophotometer at 517 nm (Jasco, USA, V-730 UV-Vis spectrophotometer). The negative control was absolute distilled water. The scavenging activity of DPPH was determined as follows:

$$DPPH \; scavenging \; activity \; (\%) = \frac{(\text{Ao}-\text{A1})}{\text{Ao}} \text{ x } 100$$

Where A_1 is the absorbance of test solutions, and A_0 is the absorbance of the control.

Ascorbic acid was used as a positive control. Measurements were made in triplicate. The concentration required for a 50% inhibition of DPPH (IC_{50}) was then calculated by plotting the percentage of residual DPPH against the sample concentration (Sánchez-Moreno, Larrauri and Saura-Calixto, 1998; Aiyegoro and Okoh, 2010; Torey *et al*., 2010; Basma *et al*., 2011; Mahdi Pour *et al*., 2012) [40, 4, 15, 23].

2.6 *In vitro* Antimicrobial activity

A broth micro-dilution experiment was used to evaluate the extracts' antimicrobial properties.

2.6.1 Inoculum Preparation

Onto nutrient agar plates were streaked bacterial isolates (Oxoid, United Kingdom) and incubated for 18–24 hours at 37 °C. Suspensions of the organisms were made in nutrient broth and incubated overnight at 37 °C as prescribed by the colony suspension method The colony suspensions in sterile saline were adjusted to 0.5 McFarland standard for the tests, and further diluted in sterile double-strength nutrient broth (2 × 10^5 CFU/mL).

2.6.2 Broth Micro-Dilution

The antimicrobial susceptibility test was conducted using the method earlier described by Agyare *et al*., (2004) [53] with modification. 100 uL of double-strength nutrient broth was dispensed into each well of the 96-well sterile plates, and estimated quantities of stock solutions of extracts were added to the wells that were suitably labeled to obtain concentrations of 50, 25, 12.5, 6.25, 3.125, and 1.6125 mg/mL. A volume of 20 µL of the freshly prepared inoculum was then transferred into each well. The microtiter plate was incubated at 37 °C for 24 hrs. Microorganism growth was determined by adding 20 µL of a solution of tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) (MTT) (5 mg/mL) to obtain a final volume of 200 µL and incubating for 30 mins. A purple color indicated that the organisms were not inhibited but no color change indicated the absence of viable cells. The MIC was determined as the least concentration which showed no color change after addition of MTT. The experiments were conducted in triplicate and results presented as mean ± SEM. Standard antimicrobial disc used was gentamycin (Akoto *et al*., 2019) [19].

2.7 Thin layer chromatographic separation of components of *A. silphioides* extract: Thin layer chromatography (TLC) was used to monitor the separation of the phytoconstituents in the methanol extract of *A. silphioides* by employing the appropriate stationary and mobile phases. The stationary phase employed was a pre-coated silica gel plate, and the solvent system ethyl acetate: hexane (2:8) was employed to achieve separation of the components on the TLC plate.
Visualization of the components was achieved under UV light (254 nm). The visualized spots of the components were marked to calculate the retardation factor (Rf) using the formula:

$$Rf = \frac{Distance\ travelled\ by\ the\ sample}{Distance\ travelled\ by\ the\ solvent}$$

2.8 Fourier Transform Infrared Spectrophotometer (FTIR) Analysis
The functional groups that were present in the extract were identified using a Fourier transform infrared spectrophotometer (FTIR) (UATR Two, PerkinElmer). After scanning the area between 400cm^{-1} and 4000cm^{-1}, baseline correction was performed.

2.9 Gas Chromatography-Mass Spectrometer (GC-MS) Analysis: Gas chromatography (PerkinElmer GC Clarus 580 Gas Chromatograph) linked to Mass Spectrometer (PerkinElmer Clarus SQ 8 S) and connected to a tubular column (30 0.25 m ID 0.25 m DF) fused with ZB-5HTMS (5% diphenyl/95% dimethyl polysiloxane) was used to examine the extract to identify the phytochemicals contained in the extracts. The oven's temperature was programmed to begin at 100 °C (isothermal for 2 min), climb by 10 °C/min to 200 °C, and then decrease by 5 °C/min to 280 °C. An electron ionization system with an ionization energy of 70 eV was run in electron impact mode for GC-MS detection. With an injection volume of 1 µl and a steady rate of flow of 1 ml/min, helium gas (99.9999%) was utilized as the carrier gas. The ion-source temperature was 220 °C, while the injector temperature was maintained at 250 °C. At 70 eV, a scan interval of 1s, and an MS scan from 45 to 500 Da were used to capture the mass spectra. The entire running duration for the GC/MS was 43 min, and the solvent delay ranged from 0 to 3 min.

2.10 Statistical Analysis
The results were processed and analyzed using Microsoft Excel 2016 and GraphPad Prism 5 software version 8.02. (GraphPad Software, San Diego, CA, USA). Analysis of variance (ANOVA) was employed to assess the significant difference between variables.

3. Results and Discussion

3.1 Percentage yield
The percentage yield (%) of the methanol extract was 12.83, which means that the extraction using a polar solvent produced a quantifiable amount of extract. The yield was calculated in relation to the amount of pulverized sample used for the extraction.

3.2 Phytochemical screening
The results of the phytochemical screening are presented in Table 2. The methanol extract of *A. silphioides* contained phenols, steroids, tannins, terpenoids, flavonoids, phytosterols, and polyphenols. Carotenoids, quinones, saponins, glycosides, and alkaloids were absent in the extract or couldn't be detected. Flavonoids, terpenoids, steroids, phenolic acids, and alkaloids are examples of secondary metabolites of plants that have demonstrated a variety of pharmacological effects including anticancer, anti-inflammatory, wound healing, antibacterial, antiplasmodial, and antioxidant activities (Boniface *et al*., 2016) [16].
According to Zhang *et al*., (2015) [51], polyphenols and carotenoids are the two main types of antioxidant phytochemicals that contribute mostly to the antioxidant properties of foods/plants. Flavonoids and their derivatives have antitumor, antioxidant, anti-inflammatory, anti-diabetic, and enzyme inhibition activities (Nile *et al*., 2014) [29]. Research has revealed that polyphenols are involved in anti-radical activity, and benzopyran is also involved in cytotoxicity (Hasan *et al*., 2014) [20].

Table 1: Phytochemicals in the pulverized sample and methanol extract of the leaves of *A. Silphioides.*

Phytochemical	Methanol Extract
Alkaloid	-
Phenol	+
Glycosides	-
Saponins	-
Steroids	+
Tannins	+
Terpenoids	+
Flavonoids	+
Quinones	-
Phytosterols	+
Polyphenols	+
Carotenoids	-

KEY: (+) = Secondary metabolites present; (-) = Secondary metabolite absent or not detected.

3.3 Identification of phytoconstituents of the methanol extract by GC-MS
Analysis of the chromatogram and mass spectra led to the identification of various phytochemicals which have been reported in table 2. Tentative identification of the compounds was done by comparing the fragmentation patterns of the phytoconstituents with those of authentic standards from the National Institute of Standards and Technology (NIST) library. Confirmation of the compounds was done by employing the similarity index.

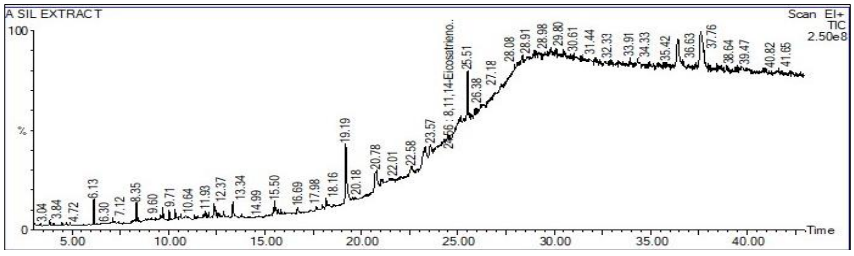
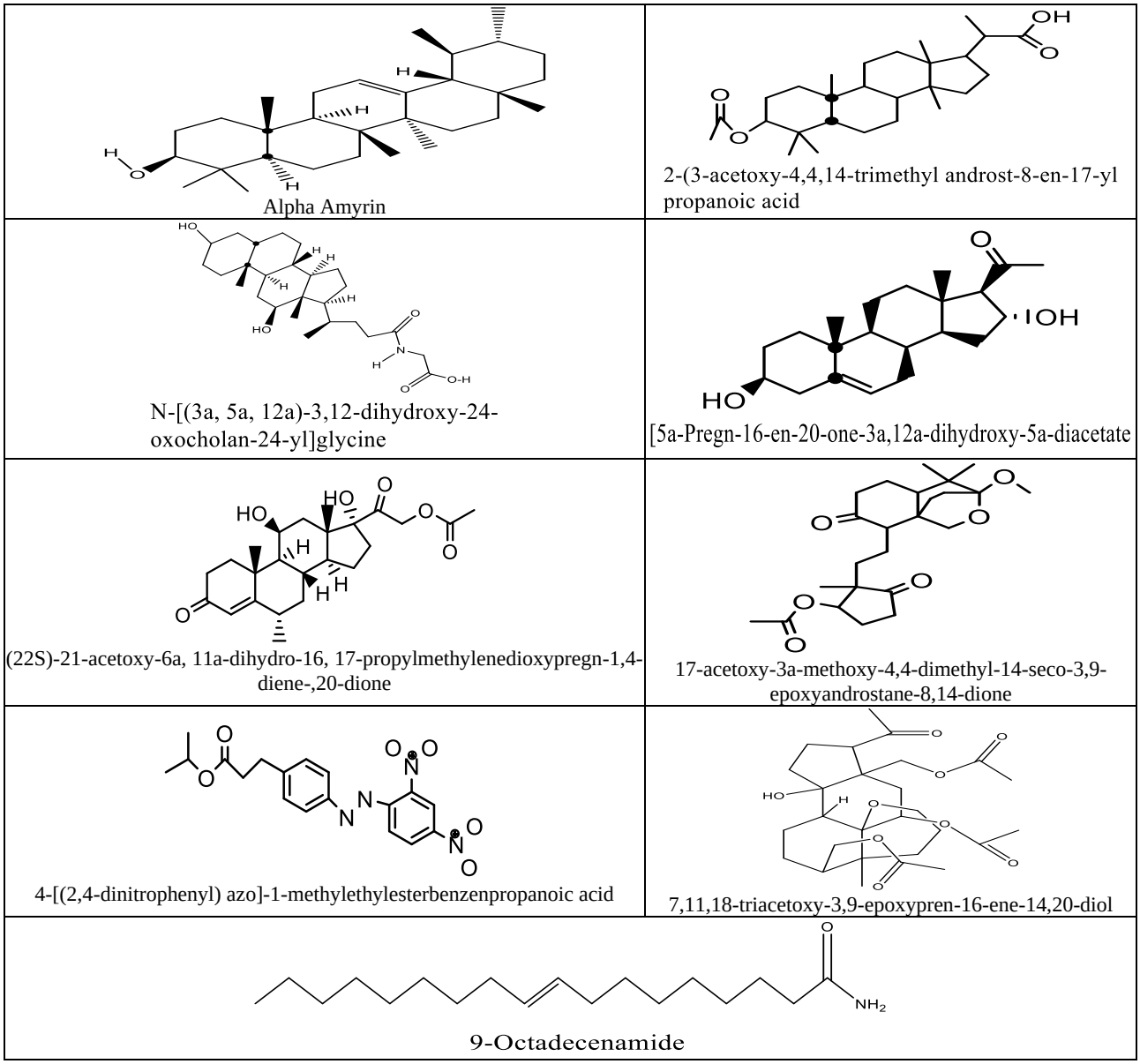


Fig 1: GC-TIC chromatogram of the methanol extract of *A. silphioides.*

Table 2: Phytochemicals identified in the methanol extract of *A. silphioides* using GC-MS.

Compounds	Retention time (min)	Percent Abundance	Similarity Index
9-Octadecenamide	19.189	0.976	94.7
2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl) propanoic acid	29.805	35.325	97.2
17-acetoxy-3a-methoxy-4,4-dimethyl-14-seco-3,9-epoxyandrostane-8,14-dione	34.333	3.409	91.1
4-[(2,4-dinitrophenyl)azo]-1-methylethylesterbenzenpropanoic acid	35.415	1.443	91.6
N- [(3a, 5a, 12a)-3,12-dihydroxy-24-oxocholan-24-yl] glycine	35.782	1.307	97
α-Amyrin	36.424	6.085	91.3
5a-Pregn-16-en-20-one-3a,12a-dihydroxy-5a –diacetate	37.322	1.078	93.1
(22S)-21-acetoxy-6a, 11a-dihydro-16, 17-propylmethylenedioxypregn-1,4-diene-,20-dione	38.367	0.972	99.7
7,11,18-triacetoxy-3,9-epoxypren-16-ene-14,20-diol	39.706	0.912	99.4

The TIC chromatogram of the GC showed the presence of nine major peaks at various retention times. The compounds identified to be present are presented in Table 2. These compounds are likely responsible for the bioactivities of the methanol leaf extract of *A. Silphioides. For instance, α*-tocopherol is known for its antioxidant activity (Winbauer *et al*. 1999) [48]. 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl) propanoic acid is known to have antimicrobial activity against Staphylococcus spp. (Saranya R., *et al*. 2015) [41]. Again, α-Amyrin has been confirmed to have antioxidant, anti-inflammatory, anticonvulsant, and hepatoprotective effects. (Oliveira, R., 2022 & Seki H. *et al*. 2008) [32, 43]. The bioactivity of N- [(3a, 5a, 12a)-3,12-dihydroxy-24-oxocholan-24-yl] glycine is known to have antimicrobial activity (Wyson, J., *et al*. 2016) [50]. 5a-Pregn-16-en-20-one-3a,12a-dihydroxy-5a –diacetate is known to have antimicrobial activity. (Matys E., *et al*., 2019) [24]. Antimicrobial activity of 4-[(2,4dinitrophenyl) azo]-1-methylethylesterbenzenpropanoic acid is prominent against some bacteria (Foo, L., *et al*., 2017) [19].



*All the chemical structures were drawn with CHEMDRAW Professional 16.0

Fig 2: Chemical structures of the compounds identified in the methanol extract of *A. Silphioides.*

GC-MS analysis on medicinal plants including ethanolic extracts of *Pistia stratiotes L.* and *Eichhornia crassipes* (Tyagi *et al*., 2017) [46], and leaf extract of *Eclipta prostrata* L. (Wyson *et al*., 2016) [50], have identified various biologically active compounds responsible for various pharmacological activities.

3.4 FTIR Analysis of the Methanol Leave Extract of *A. Silphioides*

The extract obtained from the cold maceration extraction was subjected to FTIR analysis and the results is depicted in Figure 3.

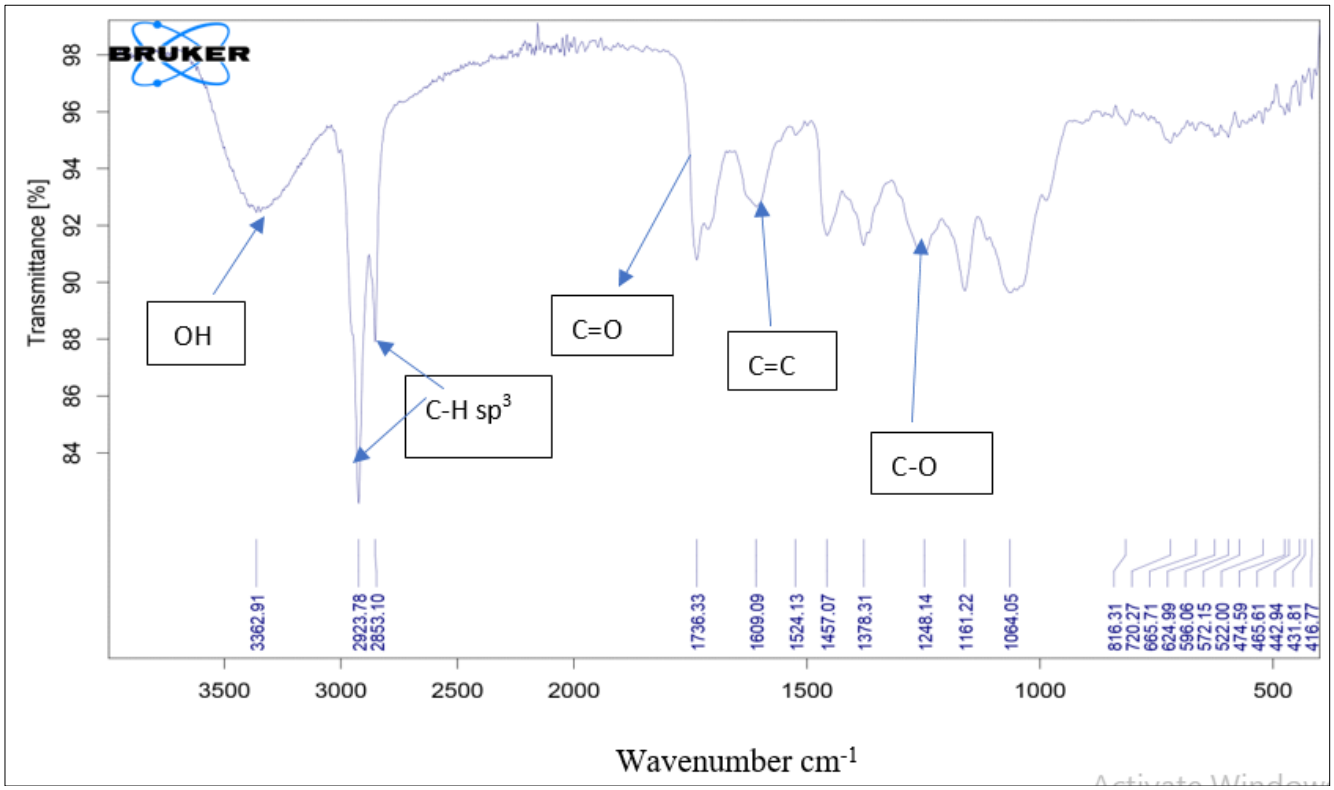


Fig 3: The FTIR spectrum of the methanol extract of *A. silphioides.*

A summary of the peak interpretation of the FTIR spectrum of the methanol extract of *A. silphioides* is given in Table 3. The methanol extract contains C-H sp^3 stretch appearing at peak at 2923.78 - 2853.10 cm^{-1}. A C=O carbonyl peak was displayed at 1738.33 cm^{-1} and an OH peak at 3362.91 cm^{-1}. There was a C=C peak at 1609.09 cm^{-1} and a promising peak at 1248.14 cm^{-1} showing a C-O stretch. The extract's FTIR spectrum demonstrated the presence of functional groups found in the phytochemicals that the GCMS determined to be present in the extract. For example, in 9-octadecenamide there is the presence of C=O which has a peak around 1680 cm^{-1} to 1780 cm^{-1}.

Table 3: Peak interpretation of the FTIR spectrum of the methanol extract of *A. silphioides*.

Functional groups	Peaks value (cm^{-1})
Sp^3 C-H stretch (aliphatic)	2800-2960
C=O carbonyl	1650-1750
OH stretch	3400-3600
C=C stretch	1600-1680
C-O stretch	1100-1300

3.5 *In vitro* antioxidant activity of the methanol extract

The antioxidant activity of the methanol extract of *A. silphioides* was evaluated using DPPH radical scavenging activity, and hydrogen peroxide scavenging activity.

3.5.1 DPPH radical scavenging activity

The antioxidant activity of the methanol leaf extract of *A. silphioides* was assessed in comparison to a reference medication (ascorbic acid) using DPPH radical scavenging activity. The ability of the methanolic extract of *A. silphioides* to mop up free radicals in people and animals was specifically assessed using the extract's DPPH scavenging activity.

Table 4: DPPH scavenging activity of the methanol extract of *A. silphioides* and ascorbic acid standard.

Concentration (µg/ml)	Methanol extract		ascorbic acid	
	Mean Absorbance ±S.Em	% Scavenging	Mean Absorbance ± SEM	% Scavenging
100	0.66±0.040^b	57.32	0.74±0.040^a	65.83
80	0.62±0.045^b	52.68	0.73±0.010^a	58.22
60	0.56±0.039^b	34.43	0.71±0.040^a	56.96
40	0.46±0.010^b	32.38	0.53±0.046^a	51.88
20	0.41±0.040^b	31.51	0.49±0.063^a	47.05

Table 4 presents the scavenging activity of the extract and the ascorbic acid. Figure 4 depicts the percentage scavenging activity of the methanol extract and the standard ascorbic acid over a range of concentrations. The activity of both the methanol extract and the ascorbic acid increased with increasing concentrations. At all concentrations, the standard drug exhibited significantly higher percentage inhibition than the methanol extract. The results indicate that the extract was able to mop up the free radicals by transferring a hydrogen atom to the DPPH to make it a stable compound with yellow coloration.

The IC_{50} of the methanol extract and the standard ascorbic acid are presented in Table 5. The IC_{50} value is a parameter

widely used to measure the antioxidant activity of test samples (Sanchez-Moreno *et al.*, 1998) [40]. It is calculated as the concentration of antioxidant needed to decrease the initial DPPH concentration by 50%, hence, the lower the IC_{50} value the higher the antioxidant activity. Comparing the IC_{50} values of the methanol extract (47.66) and the ascorbic acid (40.29), the ascorbic acid would have higher antioxidant activity than the extract. For the IC value, when compared to the methanol extract, ascorbic acid was found to be significantly greater (P 0.0039).

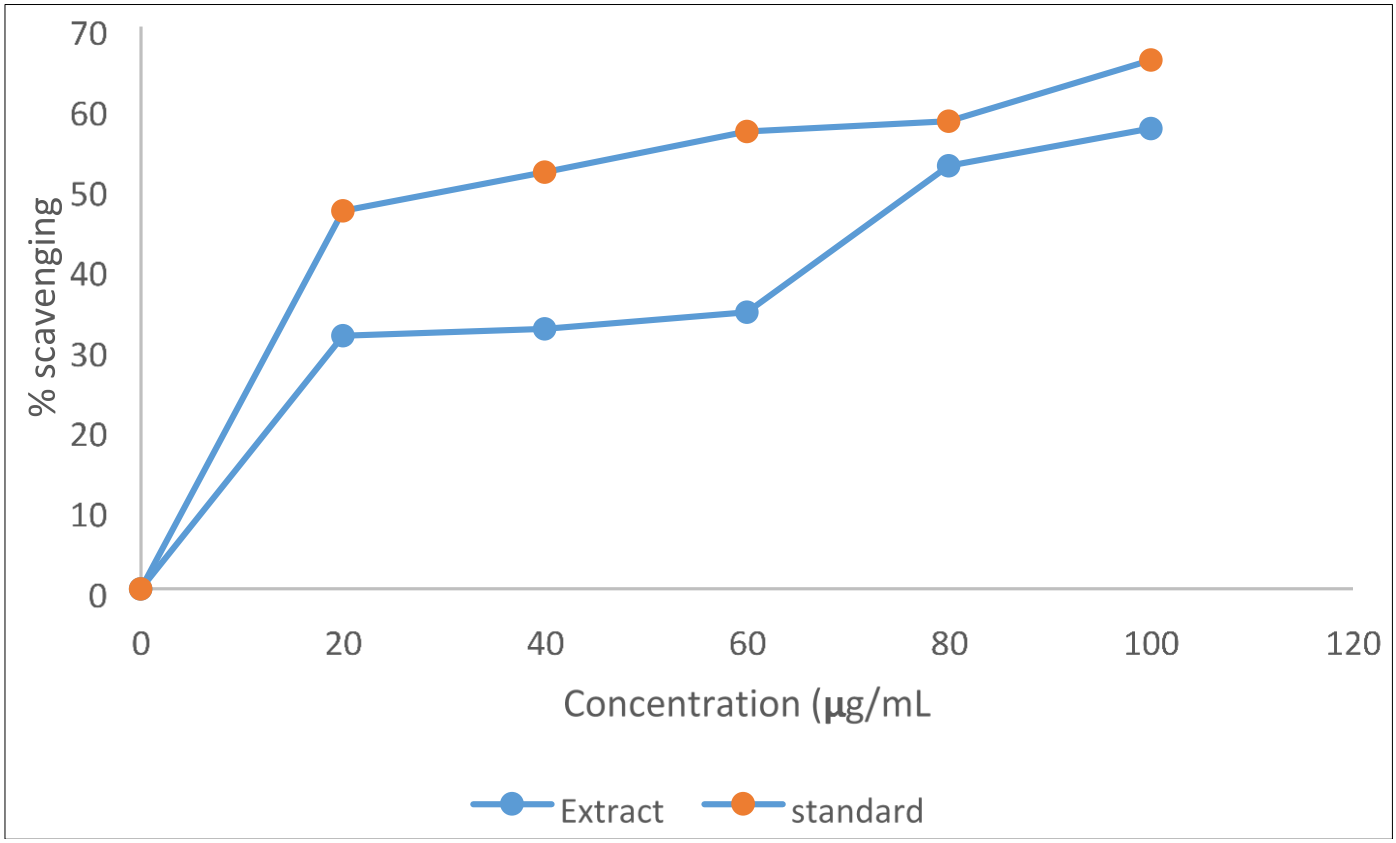


Fig 4: Comparative radical scavenging activity of the methanolic extract of *A. silphioides* and the ascorbic acid standard.

Table 5: The IC_{50} for DPPH and H_2O_2 scavenging capacity for the methanol extract, ascorbic acid, and Gallic acid.

Sample	IC 50 (µg/ml)	
	DPPH	H2O2
Methanol extract	47.66±0.61	39.72±0.004
Ascorbic acid	40.29±0.62	N/A
Gallic acid N/A 32.62±0.001		

Key: N/A = Not Applicable

4.5.2 Hydrogen peroxide scavenging activity of methanol extract

The hydrogen peroxide scavenging activity was used to determine the scavenging activity of the methanol extract of the leaves of *A. silphioides.* Gallic acid was used as the standard drug. The results are presented in Table 5.

In the results obtained, the absorbance of the methanol extract and standard drug increased with increasing concentration. The antioxidant activity of a drug is measured by its IC_{50} value, and the lower the value, the greater the antioxidant activity. The antioxidant activity of the Gallic acid is greater than that of the methanol extract when the IC_{50} values of the extract and Gallic acid are compared (Table 5 and Table 6).

Due to its strong capacity to penetrate biological molecules, hydrogen peroxide is a significant reactive oxygen species. It is created when superoxide dismutase produces superoxide radicals, which are then transformed into hydroxyl radicals (OH) by glutathione and catalase in the presence of copper or iron.

Table 6: Hydrogen peroxide scavenging activity of methanol extract and Gallic acid standard.

Concentration (µg/ml)	Methanol Extract		Gallic Acid	
	Mean Absorbance ±SEM	% Scavenging	Mean Absorbance ±SEM	% Scavenging
1000	0.69±0.0005[b]	78.35	0.64±0.0001[a]	71.69
800	0.69±0.0001[b]	77.53	0.60±0.0001[a]	67.64
600	0.54±0.0005[b]	60.49	0.54±0.0001[a]	60.34
400	0.42±0.0005[b]	46.93	0.35±0.0001[a]	39.77
200	0.27±0.0005[b]	30.34	0.32±0.0001[a]	36.07

The hydroxyl radical (OH.) is one of the oxygen radicals, and it is the most reactive radical that can harm macromolecules in the biological system like proteins, carbohydrates, and lipids (Ligouri *et al.*, 2018) [21]. Antioxidant activity was observed in the methanol extract. It was found that the Gallic acid had a significantly higher IC50 than the methanol extract ($P < 0.0045$).

Figure 5 depicts the H_2O_2 scavenging activity of the methanol extract and Gallic acid. The antioxidant activity of the extract could be due to the presence of α-Amyrin (6.085%), and other flavonoids and polyphenols in the extract since they have been found to have antioxidant properties.

In vitro antioxidant studies of some medicinal plants have also proven that medicinal plants possess antioxidant properties (Acheampong *et al.*, 2018; Akoto *et al.*, 2020; Mahdi-Pour *et al.*, 2012) [7, 9, 23]

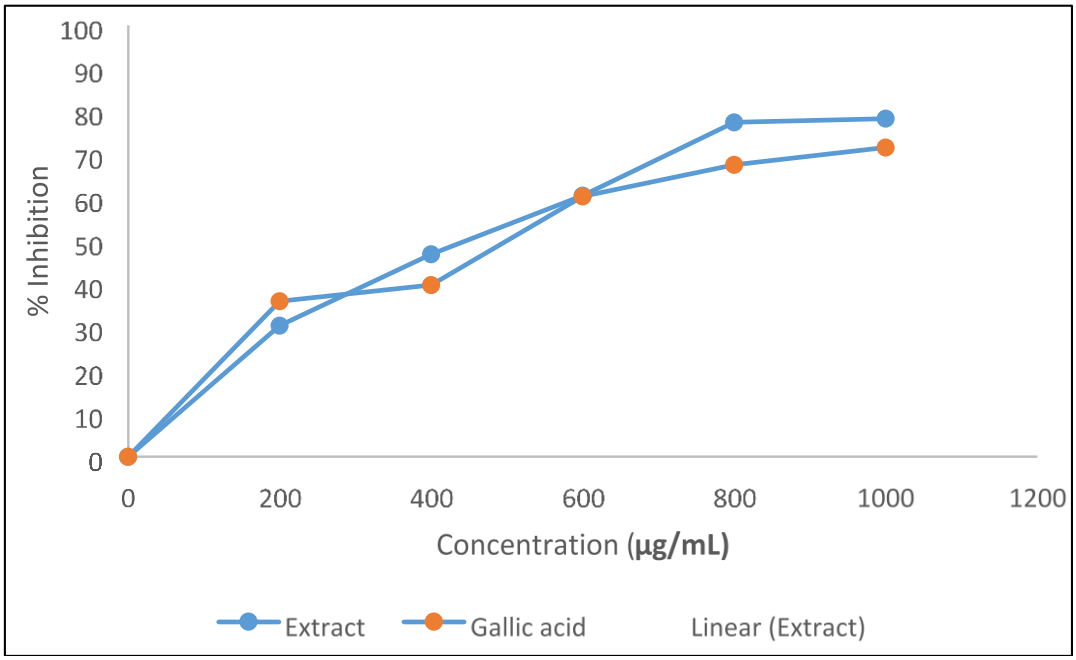


Fig 5: H_2O_2 scavenging activity of the methanol extract of *A. silphioides* and Gallic acid.

3.5.3 *In vitro* anti-helminthic activity of the methanol extract

Table 7 summarizes the anti-helminthic activities of the standard drug and the methanol extract. *Eudrilus eugeniae* (Earthworms) were used to test the methanol extract's anti-helminthic activity because of its anatomical and physiological similarity to human intestinal roundworm parasites and the ease of availability.

Table 7: The mean paralysis and death times of the earthworm (*Eudrilus eugeniae*).

Treatment	Mebendazole		Methanol extract	
Concentration (mg/ml)	Mean Paralysis Time (min) ±SEM	Mean Death Time (min) ±SEM	Mean Paralysis Time (min) ±SEM	Mean Death Time (min) ±SEM
20	27.15±15.67 [b]	82.03±47.37 [b]	20.23±0.24 [a]	53.76±2.89 [a]
10	34.19±19.74 [b]	126.07±72.55 [b]	37.10±0.73 [a]	91.00±7.22 [a]
5	69.60±40.18 [b]	170.00±98.15 [b]	52.54±1.69 [a]	141.00±5.09 [a]
2.5	139.33±80.44 [b]	184.33±106.42 [b]	59.11±0.93 [a]	177.07±0.24 [a]
1.25	150.00±80.60 [b]	223.00±128.74 [b]	78.07±2.83 [a]	210.00±8.51 [a]
Normal saline	-	-	-	-

Table 7 presents the mean time for the paralysis and death of the worm with their corresponding concentrations. As the concentration increased, both the death and paralysis time of the worm decreased. Hence, anti-helminthic activity increases with increasing concentration of extract. Similarly, the anti-helminthic activity of the standard drug increased with increasing concentration of the drug. The paralysis time of the extracts ranged from 20.23 to 78.07 mins between the concentration range of 1.25 to 20 mg/mL. Within the same concentration range, the paralysis time of the standard drug ranged from 27.15 to 150 mins. The shorter the paralysis time, the more potent the sample. At the maximum concentration of 20 mg/mL the methanol extract exhibited a higher anti-helminthic activity than the standard drug as it recorded lower paralysis and death times. At the least concentration of 1.25 mg/mL, the extract was almost twice as potent as the mebendazole in paralyzing the worms, and more active than the mebendazole in terms of killing the worms. As the concentration of the extract and mebendazole increased the death time for the worms also reduced. This means the higher the concentration the lesser the death time of the worm. The methanol extract exhibited anti-helminthic activity. The methanol extract was found to have significantly more anti-helminthic activity than the mebendazole (P<0.0001 and 0.0007).

Anthelmintics are medications that help to get rid of parasitic worms (helminths) and other internal parasites by killing or stunning them without harming the host (Rajani *et al*., 2013) [39]. The extract exhibited this activity by paralyzing the worm and even killing them at a faster rate than the standard drug. Phytochemicals such as tannins and terpenoids present in the extract could be responsible for the high activity of the extract since they have been found to possess anti-helminthic activity (Raut *et al*., 2009) [39]. Some medicinal plants including *Ocimum bacilicum* (Akoto *et al*., 2020b) [7, 9], *Sclerocarya birrea* (Akoto *et al*., 2020a) [7, 9], and a combination of four medicinal plants (Padmanabhan and Jangle, 2012) [33] have been proven to have anti-helminthic properties.

3.5.4 *In vitro* antimicrobial assessment of the methanol extract: The technique utilized to determine the minimum inhibitory concentration (MIC) was the microwell broth dilution. The concentrations of the extract range from 50 mg/mL to 1.25 mg/mL, and $5x10^{-3}$ mg/mL for the standard drug (gentamicin). The results of the MIC and MBC are presented in Table 8.

Table 8: The MIC of the methanol extract of *A. silphioides* and standard drug (Gentamicin).

Test organisms	Minimum Inhibitory Concentration (mg/ml) ± SEM	
	Methanol extract	Gentamicin
C. albicans	25 ± 0.013	$5x10^{-3}$ ± 0.011
E. coli	25 ± 0.010	$5x10^{-3}$ ± 0.012
S. aureus	12.5 ± 0.011	$5x10^{-3}$ ± 0.012
S. pyogenes	25 ± 0.015	$5x10^{-3}$ ± 0.009
S. typhi	25 ± 0.012	$5x10^{-3}$± 0.014
P. aeruginosa	50 ± 0.020	$5x10^{-3}$± 0.011

The antimicrobial susceptibility test was conducted using the method earlier described by Agyare *et al*., 2004 [53] with slight modification. The results of the antimicrobial test are presented in Table 8. The minimum inhibitory concentration (MIC) is the minimum concentration of antimicrobial agent that inhibits the visible growth of microorganisms after overnight incubation, and the minimum bactericidal concentration (MBC) is the minimum concentration of antimicrobial agent that inhibits the growth of the organism. The methanol extract exhibited antimicrobial activity and recorded various MIC at different concentrations. The MIC of the methanol extract against the micro-organisms ranged from 12.5 mg/mL to 50 mg/mL, with *S. aureus* being the most susceptible and *P. aeruginosa* being the least susceptible. A constant MIC value of $5x10^{-3}$ mg/mL was obtained for all microorganisms by gentamicin. This implies that the methanol extract of the leaves of *A. silphioides* is potent against the microorganisms but less potent than gentamicin, the standard drug. The extract showed antimicrobial activity against all tested organisms, indicating that the extract of the plant can be a source of a potential antimicrobial drug. It may also show antimicrobial activity against other microbes that cause a variety of diseases. The compounds 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl) (35.325 % abundance), N- [(3a, 5a, 12a)-3,12-dihydroxy-24-oxocholan-24-yl] glycine (1.307%), 5a-Pregn-16-en-20-one-3a,12a-dihydroxy-5a–diacetate (1.078%), and 4-[(2,4-dinitrophenyl) azo]-1-methylethylesterbenzenpropanoic acid (1.443%) with known antimicrobial properties, could be responsible for the antimicrobial activity of the extract.

Many plants, including *A. silphioides*, have been used for decades to treat bacterial infections, and some have been proven scientifically to possess antimicrobial activities (Akoto *et al*., 2019; Akoto *et al*., 2020 [19, 7, 9]

Conclusion

Some of the pharmacological activities of methanol extract of *A. silphioides* have been assessed, and its phytoconstituents screened. The methanolic extract of *A. silphioides* exhibits potent anti-helminthic, antimicrobial, and antioxidant activities, and the extract could be a potential therapeutic agent for the treatment of helminthiasis, microbial infections, and oxidative stress diseases. The results obtained validate the use of *A. silphioides* leaf as an anti-helminthic and wound-healing agent by the indigenous people. Phytochemical screening revealed the presence of Phenols, steroids, tannins, terpenoids, flavonoids, phytosterol, and polyphenols in the methanolic extract of *A. silphioides*. GC-MS analysis identified nine phytoconstituents including 9-Octadecenamide, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl) propanoic acid, 17-acetoxy-3a-methoxy-4,4-dimethyl-14-seco-3,9-epoxyandrostane-8,14-dione, αAmyrin, 4-[(2,4-dinitrophenyl) azo]-1-methylethylesterbenzenpropanoic acid, 5a-Pregn-16en-20-one-3a,12a-dihydroxy-5a–diacetate in the methanol leaf extract. Isolation, purification, characterization, structural elucidation, and bioactivity determination of the isolates of the methanolic leaf extracts could lead to finding lead compounds for helminthiasis, one of the neglected tropical diseases, and other diseases related to oxidative stress and microbial infections.

Data Availability

All the data has been included in the manuscript.

Conflict of Interest

The authors have no conflict of interest (financial, professional, or personal) to declare.

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