



Gas Chromatography-Mass Spectrometry Analysis of the Aqueous Fraction of *Caralluma dalzielii* for Antischistosomal Activity

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Abstract

Schistosomiasis remains a public health burden in tropical and subtropical countries with 90% of the cases occurring in Africa. Treatment of the disease has relied on a single drug praziquantel (PZQ) for more than one generation, thus increasing the possibility of drug resistance. The majority of the pharmaceutical industry rely on medicinal plants as their raw materials. *Caralluma dalzielii* belonging to the family Asclepiadaceae is well known for its Antischistosomal activity. This work is aimed to determine the phytochemical profile of the aqueous fraction of *C. dalzielii* using GC-MS analysis. Using conventional techniques, GC-MS Analysis was performed on GC-MS-QP2010 Plus Shimadzu. The component spectrums were compared to the database of known component spectrums kept in the GC-MS library. Eleven bioactive compounds with antihelminthic activity were identified by GC-MS analysis, originating from distinct peaks. These compounds include Octadecanoic acid, 1, E-8, Z-10-Hexadecatriene, 9, 12, Octadecanoic acid, Diethylene, and Ricinoic acid. The aqueous fraction of *C. dalzielii*'s GC-MS profile showed the existence of metabolites with significant pharmacological characteristics. Hence, the plant extract's antischistosomal actions may be caused by the presence of these phytochemical metabolites.

Keywords: Schistosomiasis, *Caralluma dalzielii*, Antischistosomal, GC-MS analysis, Bioactive compounds

Introduction

Schistosomiasis is a disease caused by parasitic flatworms called schistosomes is a significant public health issue that requires preventive chemotherapy for over 240 million individuals, more than 90% of whom reside in sub-Saharan Africa (Shady, 2024; Ally et al., 2024). Treatment of the disease relies on conventional drugs and is effective in the treatment of the disease, but has achieved very little progress in the treatment of schistosomiasis due to varying drug responses (Condeng et al., 2024; Bergquist et al., 2017). Praziquantel is the most effective drug against all stages of human schistosomiasis and is not effective in juvenile stages (Kabuyaya et al., 2018). However it has been in use for more than twenty years thus increasing the possibility of the emergence of drug resistance (Waechter et al., 2023). Plants are the greatest supply of pharmaceutical intermediates, food supplements, ancient and contemporary medicine's pharmaceuticals, and chemical entities for synthetic drugs (Chaachouay & Zidane, 2024; Basu et al., 2023). It has been reported that thousands of plant species have therapeutic properties, and using various plant components to treat particular illnesses has been practised since ancient times. Certain chemical compounds found in plants have a specific physiological function, which gives them their therapeutic value (Zhou et al., 2023). It is generally accepted that one useful method for finding novel, potent plant drugs is phytochemical research based on ethnopharmacological studies (Schultz & Garbe, 2023). *C. dalzielii* is a succulent herb occurring wild in the sehelian region of West Africa. It has antispasmodic, antidiabetic, analgesic, anti-inflammatory, anthelmintic, and ameliorative properties and is utilized in traditional medicine (Tanko et al., 2013; Umar et al., 2013; Marinella et al., 2005). The plants grow up to 40 cm tall, perennial, erect, and sparsely branched. They have green stems and quadrangular branches that are strewn with dark reddish-purple star-shaped blooms. In Northern Nigeria, the Fulani people refer to it as Gubehi, and the Hausa people name it Karan Masallaci (Umar et al., 2013). The aqueous extract of *C. dalzielii* contains phytochemicals such as Saponin, tannins, terpenoids, steroids and cardiac glycosides and possesses antischistosomal activity against the cercarial and adult *S. mansoni* invitro (Mohammed et al., 2021). An analytical method Gas Chromatography-Mass Spectroscopy (GC-MS) is used to identify the chemicals

contained in a plant sample. The work is aimed to identify phytochemical compounds present in *C. dalzielii* aqueous extract.

Materials and Methods

Healthy plants of *C. dalzielii* were collected from Saye in Zaria Local Government of Kaduna state where the plants grow abundantly. The plant samples were collected in plastic bags and brought to Ahmadu Bello University's Herbarium Department in Zaria for identification and verification. *C. dalzielii* was given voucher number ABU01408. Plant samples were cleaned and washed under running tap water and air dried at room temperature. The stem and root of *C. dalzielii* were cut into small bits with the aid of a kitchen knife to aid in drying. To standardize the particle size, the fresh, dried plants were ground into a powder using a pestle and mortar and then run through a 0.5 mm mesh screen. After that, the powder was maintained at room temperature in glass bottles (Teresa, 2018). The distilled water of 1000 ml was used to dissolve 100g powder of *C. dalzielii*. The mixture was filtered through Whatman filter paper No. 1 after being left at room temperature for 48 hours. The concentrate was made at 40°C in a rotary evaporator. To extract the solvent from the extract, the filtrate was dried at 25 °C in a water bath (Teresa, 2018). Gas chromatography-mass spectrometry (GC-MS) Analysis was carried out on GC-MS-QP2010 PLUS SHIMADZU. A 30 m x 0.25 mm Perkin Elmer Elite-5 capillary column with a 0.25 mm film thickness made of 95% diethyl polysiloxane was utilized. Helium was the carrier gas, flowing at 0.5 millilitres per minute. A sample injection volume of 1 µl was used, and the inlet temperature was kept at 250 °C. The oven temperature was set to start at 80 °C for four minutes, rise to 200 °C, and then decrease by 20 °C over the course of five minutes to reach 280 °C. The run took 25 minutes in total. The source temperature was kept at 180 °C, while the Mass Spectrometry transfer line was kept at 200 °C. Using electron impact ionization at 70 eV, GCMS data was processed, and ion count (TIC) was used to assess the data for compound identification and quantification. The component spectrums were compared to the database of known component spectrums kept in the GC-MS library.

Results

A total of eleven bioactive compounds were identified from the Gas chromatography-mass spectroscopy (GC-MS) analysis of aqueous extract of *C. dalzielii*. The chromatogram is presented in Fig. 1

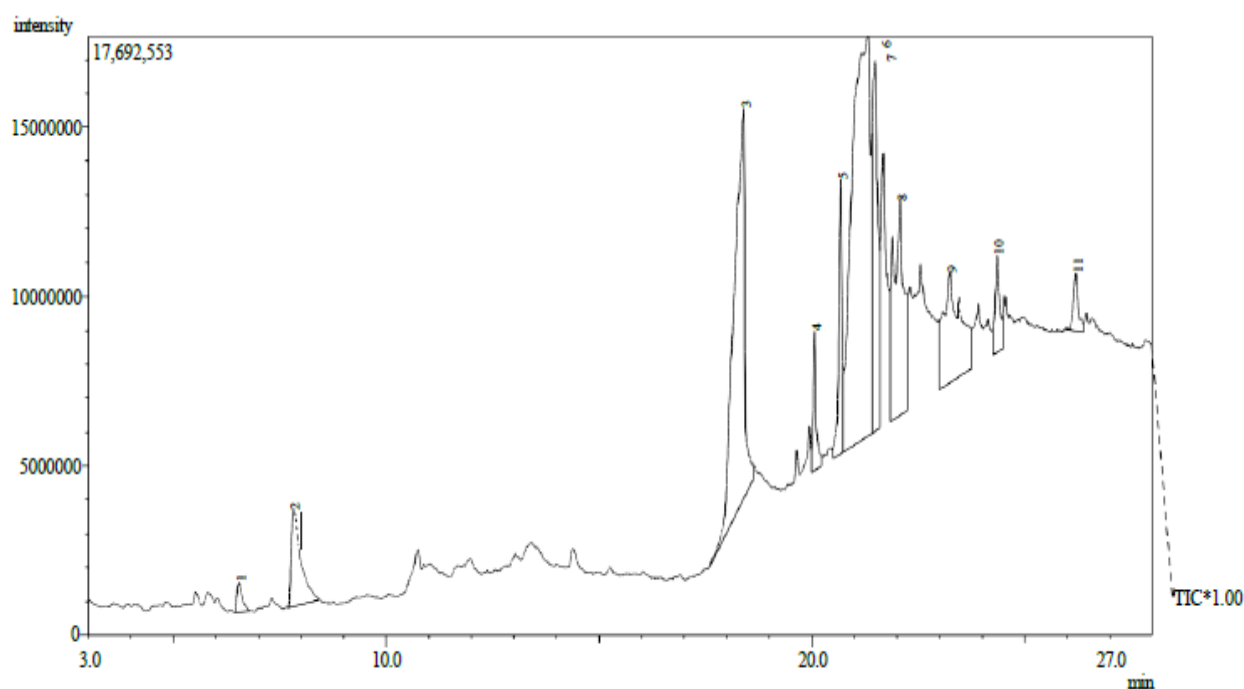


Fig. 1 GC-MS chromatogram of aqueous extract of *C. dalzielii*

The bioactive compounds present in the GC-MS analysis carried on aqueous extract *C. dalzielii* include 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 5-hydroxymethylfurfural, Hexadecanoic Acid, 9,12-octadecadienoic acid, 1,E-8,Z-10-hexadecatriene, 9-octadecenoic acid, Octadecanoic Acid, 1,E-11,Z-13-

Octadecatriene, Ricinoic Acid, Trans-13-decosenoic acid, 9,12-Octadecadienoyl chloride, (ZZ). Their Peaks, Retention time (RT), Area (%), Mass fragmentation, Molecular formula and name of the compounds are presented in Table 1.

Table 1: Bioactive compounds of aqueous extract of *Caralluma dalzielii*

L	RT (Min)	Area (%)	Mass fragmentation	Molecular formula	Name of compound
1	6.5	0.72	38, 43, 58, 72, 85, 101, 115, 130, 144	C ₆ H ₈ O ₄	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one
2	7.8	4.39	14, 27, 41, 53, 69, 81, 97, 109, 125, 126	C ₆ H ₆ O ₃	5-hydroxymethylfurfural
3	18.4	20.4	27, 41, 43, 60, 73, 85, 98, 115, 129, 157, 171, 185, 213, 256	C ₁₆ H ₃₂ O ₂	Hexadecanoic acid
4	20.1	2.1	39, 41, 55, 67, 81, 95, 109, 123, 135, 149, 163	C ₁₉ H ₃₂ O ₂	9,12-octadecadienoic acid
5	20.1	4.5	40, 41, 55, 67, 81, 96, 109, 121, 135, 149, 163, 220	C ₁₆ H ₂₈	1,E-8,Z-10-hexadecatriene
6	21.3	34.6	40, 41, 55, 69, 83, 97, 112, 127, 151, 165, 180, 194, 207, 221, 235, 246, 264, 282	C ₁₈ H ₃₄ O ₂	9-octadecenoic acid
7	21.4	9.9	27, 41, 60, 73, 85, 98, 115, 129, 143, 171, 185, 199, 227, 241, 284	C ₁₈ H ₃₆ O ₂	Octadecanoic Acid
8	22.1	10.0	41, 55, 67, 81, 95, 110, 121, 135, 147	C ₁₈ H ₃₂	1,E-11,Z-13-Octadecatriene
9	23.3	8.9	27, 41, 55, 69, 81, 97, 113, 137, 148, 166, 184	C ₁₈ H ₃₄ O ₃	Ricinoic Acid
10	24.4	2.1	40, 41, 27, 41, 55, 69, 81, 97, 98, 112, 137, 152	C ₂₂ H ₄₂ O ₂	Trans-13-decosenoic acid
11	26.2	1.3	27, 41, 55, 67, 81, 95, 123, 135, 151	C ₁₈ H ₃₁ ClO	9,12-Octadecadienoyl chloride, (ZZ)

The chemical constituents with their molecular formula, retention time (RT), mass fragment and molecular formula name of the compounds are presented in Table 1. The following are the compounds present in GC-MS analysis of aqueous fraction of *C. dalzielii*. The bioactive compounds include: **Peak 1** (Retention Time: 6.5 minutes, Area: 0.72%) corresponds to the compound **2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one** with a molecular formula of C₆H₈O₄. The mass fragmentation pattern includes peaks at m/z 38, 43, 58, 72, 85, 101, 115, 130, and 144. **Peak 2** (Retention Time: 7.8 minutes, Area: 4.39%) identifies **5-Hydroxymethylfurfural** with the molecular formula C₆H₆O₃. The observed mass fragmentation includes peaks at m/z 14, 27, 41, 53, 69, 81, 97, 109, 125, and 126. **Peak 3** (Retention Time: 18.4 minutes, Area: 20.4%) represents **Hexadecanoic Acid** with a molecular formula of C₁₆H₃₂O₂. The fragmentation pattern shows peaks at m/z 27, 41, 43, 60, 73, 85, 98, 115, 129, 157, 171, 185, 213, and 256. **Peak 4** (Retention Time: 20.1 minutes, Area: 2.1%) corresponds to **9,12-Octadecadienoic Acid** (C₁₉H₃₂O₂). The fragmentation pattern includes peaks at m/z 39, 41, 55, 67, 81, 95, 109, 123, 135, 149, and 163. **Peak 5** (Retention Time: 20.1 minutes, Area: 4.5%) identifies **1,E-8,Z-10-Hexadecatriene** with the molecular formula C₁₆H₂₈. The mass fragmentation shows peaks at m/z 40, 41, 55, 67, 81, 96, 109, 121, 135, 149, 163, and 220. **Peak 6** (Retention Time: 21.3 minutes, Area: 34.6%) represents **9-Octadecenoic Acid** with a molecular formula of C₁₈H₃₄O₂. The fragmentation pattern includes peaks at m/z 40, 41, 55, 69, 83, 97, 112, 127, 151, 165, 180, 194, 207, 221, 235, 246, 264, and 282. **Peak 7** (Retention Time: 21.4 minutes, Area: 9.9%) corresponds to **Octadecanoic Acid** with the molecular formula C₁₈H₃₆O₂. The mass fragmentation includes peaks at m/z 27, 41, 60, 73, 85, 98, 115, 129, 143, 171, 185, 199, 227, 241, and 284. **Peak 8** (Retention Time: 22.1 minutes, Area: 10.0%) identifies **1,E-11, Z-13-Octadecatriene** with the molecular formula C₁₈H₃₂. The fragmentation pattern includes peaks at m/z 41, 55, 67, 81, 95, 110, 121, 135, and 147. **Peak 9** (Retention Time: 23.3 minutes, Area: 8.9%) represents **Ricinoic Acid** with a molecular formula of C₁₈H₃₄O₃. The mass fragmentation pattern includes peaks at m/z 27, 41, 55, 69, 81, 97, 113, 137, 148, 166, and 184. **Peak 10** (Retention Time: 24.4 minutes, Area: 2.1%) identifies **Trans-13-Decosenoic Acid** with the molecular formula C₂₂H₄₂O₂. The observed mass fragmentation peaks are at m/z 40, 41, 27, 41, 55, 69, 81, 97, 98, 112, 137, and 152. and **Peak 11** (Retention Time: 26.2 minutes, Area: 1.3%) corresponds to **9,12-Octadecadienoyl Chloride (ZZ)** with a molecular formula of C₁₈H₃₁ClO. The fragmentation pattern includes peaks at m/z 27, 41, 55, 67, 81, 95, 123, 135, and 151.

Discussion

Among the bioactive components recovered, 9-Octadecenoic acid and Octadecanoic acid has the highest percent peak area. This compound has antischistosomal antibacterial and antifungal properties (Ghavam et al., 2021; Abdel-Hady et al., 2017; Pu et al., 2010). n-Hexadecanoic acid has antimicrobial, antioxidant, anti-fibrinolytic, and hemolytic activity, hypocholesterolemic, nematocide, pesticide, and hemolytic properties (Ganesan et al., 2022; Shaaban et al., 2021; Sharma et al., 2018). 9,12-Octadecadienoic acid has anti-nematocide, anti-inflammatory, antibacterial, anti-inflammatory and antiarthritic (Abdel Karim et al., 2021; Parthipan et al., 2015; Mathur et al., 2011). 1,E-11, Z-13-Octadecatriene has an anti-inflammatory and cancer preventive activity, nematocide, pesticide, antimicrobial, anticancer, lubricant and anti-inflammatory activities (Azeez and Mahmood, 2023; Kumaravel et al., 2019; Abiodun et al., 2014). 1, E-8, Z-10-hexadecatriene exhibit wound healing mechanisms. Trans-13-decanoic acid possesses antibacterial activity (Mathias et al., 2019). 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one affects autonomic neurotransmission, blood pressure, and body weight (Beppu et al., 2012). 5-hydroxymethylfurfural has antischistosomal activity, antihelminthic activity, Nematicidal, antimicrobial and acaricidal (Al-Quraishy et al., 2024; Vijayakumar and Thirunanasambandham, 2021; Caboni et al., 2012). 9,12-Octadecadienoyl chloride, (ZZ) has antidiabetic retinopathy, antioxidant, anti-cancerous and thyroid-inhibiting properties and antimicrobial activities (Al-Snafi, 2024; Agada et al., 2022; Kanimathi et al., 2019).

Conclusion

Phytochemical compounds that contribute to activities like anti-inflammatory, anti-helminthic, antioxidant, anticancer, and hypercholesterolemic effects were found in the GC-MS analysis. Therefore, during different phases of growth, phytochemicals have a therapeutic impact on *Schistosoma mansoni*. The bioactivity and toxicity profile of *C. dalzielii* aqueous extract need to be further investigated in order to potentially develop new medications utilizing some of the bioactive chemicals present.

Recommendations

1. To ensure the safety of these compounds for utilization, toxicity studies on *C. dalzielii* are essential given the medicinal potential of the bioactive compounds recovered.
2. It is also important to conduct toxicity studies to evaluate the potential side effects at different concentrations. Certain molecules such as 9-Octadecatrienoic acid and Octadecanoic acid, have the greatest peak areas and show potential antibacterial, antifungal and antischistosomal potentials.
3. To assess these bioactive compounds' potential for treating schistosomiasis, in vivo pharmacological investigations should be carried out on the isolated bioactive compounds.

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