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Phytochemical profiling and antimicrobial potential of the fruit and leaf extracts from *Cucumis Aculeatus*

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Abstract

Background *Cucumis aculeatus* is traditionally used to manage microbial infections such as diarrhea, wounds, and gonorrhea, yet scientific data on its phytochemical composition and antimicrobial potential remain limited. This study investigated the phytochemical profile and in vitro antibacterial and antifungal activities of *C. aculeatus* extracts.

Methods Extraction of phytochemicals was done by soaking the plant materials in cold hexane, 1:1 dichloromethane/methanol (DCM/MeOH), and 100% methanol (MeOH). Gas Chromatography–Mass Spectrometry (GC-MS) was used to identify the non-polar phytochemicals present in the extracts. The agar well disk diffusion method was used to test in-vitro antimicrobial properties of the extracts.

Results Majorly, fatty acids, esters, alcohols, and hydrocarbons were tentatively identified in crude extracts obtained from different parts of *C. aculeatus*. The fresh fruit 1:1 DCM/MeOH and dry fruit hexane extracts exhibited good inhibition against *E. coli* (ZI = 14.33 mm compared to 23.00 mm by ciprofloxacin) and *S. aureus* (ZI = 14.67 mm close to 17.33 mm by vancomycin), respectively. On the other hand, the fresh fruit methanol and dry leaves 1:1 DCM/MeOH extracts were the most effective inhibitors of *A. niger* and *A. flavus* with inhibition zones of 17.80 mm and 13.73 mm, respectively, comparatively lower than ketoconazole (26.87 mm) and voriconazole. (21.00 mm). The other extracts had low to moderate effects towards the growth of the tested bacteria and fungi strains.

Conclusion These results provide preliminary support for the traditional use of *C.aculeatus* in management of microbial infections.

Keywords *Cucumis aculeatus*, GCMS, Phytochemicals, Antimicrobial

1 Introduction

Bacterial and fungal infections represent a significant global health challenge, contributing substantially to morbidity and mortality, particularly among individuals with compromised immune systems [1]. *Escherichia coli* is a major bacterial pathogen implicated in conditions such as enteritis, urinary tract infections, septicemia, and neonatal

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meningitis [2]. Similarly, *Staphylococcus aureus* is associated with a wide range of infections that can progress to systemic disease once the organism enters the bloodstream, leading to complications such as septic arthritis, sepsis, and endocarditis [3]. Collectively, bacterial infections are estimated to cause approximately 700,000 deaths in the world annually [4].

Aspergillus niger has increasingly been implicated with cases of invasive aspergillosis, otomycosis and systemic infection. Delayed diagnosis of these infections coupled with limited therapeutic options, contribute to increased mortality rates particularly among vulnerable populations [5].

The emergence of antibiotic-resistant pathogenic microorganisms poses a substantial health challenge in the 21st century. The over usage and inappropriate use of antibiotics, combined with the inherent evolutionary adaptability of bacteria, has accelerated the development of microbial resistance [6]. Furthermore, many antifungal and antibacterial agents remain prohibitively expensive, limiting accessibility in resource-constrained settings [7]. In addition, patients may experience adverse reactions, including allergic responses, when unsuitable or excessive doses of antimicrobial drugs such as penicillin are administered [8].

Scientific evidence has consistently demonstrated that plant extracts serve as a valuable source of antimicrobial agents. A landmark example is the discovery of penicillin from molds by Alexander Fleming in 1928 [9], a significant achievement in contemporary medicine. The success of penicillin and numerous other natural products in managing microbial infections underscores the importance of continued exploration of medicinal plants as alternative therapies to address the growing challenges in infection treatment.

The wild *Cucumis aculeatus* (Fig. 1) belongs to Cucurbitaceae, a group of plants widely recognized for their medicinal properties. Members of this family have been reported to exhibit diverse therapeutic activities, including anticancer, antidiabetic, antibacterial, anti-inflammatory, and immunomodulatory effects [10]. Phytochemical investigations within Cucurbitaceae have revealed bioactive compounds such as terpenoids, glycosides, alkaloids, saponins, tannins, and carotenoids [10].

C. aculeatus has long been used in traditional medicine for the management of antimicrobial infections like abscesses, ringworms, diarrhea, wounds, and gonorrhea [11]. However, despite these ethnomedicinal applications, scientific data on its phytochemical



Fig. 1 *Cucumis aculeatus* fruit and leaves (Favour L, 2024)

composition and antimicrobial activities remain limited. Previous study by [12] reported antimicrobial activity of the plant's leaf crude extracts against bacteria; *S. aureus*, *Salmonella typhi* (Clinical isolate), *Pseudomonas aeruginosa* and *E. coli* and fungi; *Cryptococcus neoformans*, *Candida albicans*, *Microsporum gypseum* and *Trychophyton mentagrophytes*. Notably, the hexane and ethyl acetate extracts of *C. aculeatus* leaves exhibited activity against *Pseudomonas aeruginosa*. However, these studies did not include phytochemical characterization or quantitative evaluation of antimicrobial potency. Still, despite documented ethno-dietary use of the fruit in raw or cooked forms by indigenous populations, empirical validation of its therapeutic antimicrobial bioactivity remains insufficient, relative to commercial cultivars like *Cucumis sativus* and *Cucumis melo*.

The present study addresses this gap by reporting the non-polar phytochemical composition, antibacterial and antifungal activities of extracts derived from the fruit and leaves of *C. aculeatus* using different solvents. These findings provide a preliminary scientific basis for the conservation and potential utilization of this plant in the development of alternative phyto-antimicrobials.

2 Materials and methods

2.1 Plant material, reagents and test microorganisms

Fresh fruit, dry fruit and dry leaves of *Cucumis aculeatus* were used. The analytical grade hexane, dichloromethane and methanol solvents were obtained from Scielab Chemicals, Nairobi.

Test microbes used in this study included; the gram-negative, *Escherichia coli* ATCC 25922 and gram-positive *Staphylococcus aureus* ATCC 25923 bacteria and two fungi species; *Aspergillus niger* ATCC 16404 and *Aspergillus flavus* ATCC 204304.

2.2 Collection, identification and preparation of plant materials

The leaves and fruits of *C. aculeatus* were collected from Vihiga County, located in Western Kenya (Latitude: 0° 03' 60.00" N Longitude: 34° 39' 59.99" E) in the year 2024. The plant material was identified by Ms. Consolata Shilaho, a taxonomist based at Masinde Muliro University of Science and Technology. The plant species' voucher specimen (FIL/V/01/2024) was deposited at the laboratory of Botany, Department of Biological Sciences of Masinde Muliro University of Science and Technology. The fresh fruits of *C. aculeatus* were washed, cut into pieces, and blended into a fine paste. The air-dried leaves and fruits were separately cut into pieces, added to a blender and grounded individually for further investigations.

2.3 Extraction of phytochemicals from *Cucumis aculeatus*

Extraction was done sequentially through cold percolation in non-polar (hexane) and then mid-polar (dichloromethane: methanol (1:1)) and lastly polar (methanol) solvents as described by [13]. The ground plant materials were soaked in n-hexane, and the mixture was left standing for 48 h. The mixture was filtered, concentrated under reduced pressure at 40 °C using a rotary evaporator to obtain hexane extract. The residue was then soaked in dichloromethane: methanol (1:1) and finally in methanol (100%). The respective mixtures were also concentrated under similar conditions to yield

dichloromethane: methanol (1:1) and methanol extracts, respectively. The percentage yields of the individual extracts were calculated using the equation below.

$$\text{Percentage yield} = \frac{Mass\ of\ extract\ (g)}{Mass\ of\ plant\ material\ (g)} \times 100$$

2.4 GC-MS analysis of extracts of *Cucumis aculeatus*

The extracts from the leaves and fruits of *C. aculeatus*, were subjected to phytochemical profile analysis using chromatography-mass spectrometry (GC-MS) as described by [11]. The Shimadzu TQ8040 NXMass system equipped with an SH-RXi-5Sil capillary column (30 m × 0.25 mm i.d, 0.25 µm film thickness) Spectrometer was used to process the samples. The concentrated extracts were filtered through a 0.22 µm membrane filter and transferred into GC-MS vials for analysis. No chemical derivatization was performed prior to injection; therefore, the analysis was limited to volatile and semi-volatile constituents detectable under the GC-MS operating conditions. A 1 µL aliquot of the extract was injected into the GC/MS instrument using splitless mode, with helium (99.999% purity) serving as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was maintained at 250°. The GC oven was initially held at a temperature of 50 ℃ for 1 min then heated to 260 ℃ at a rate of 4 ℃ per minute and held at that temperature for 10 min. The mass spectrometer was operated under electron ionization (EI) mode at 70 eV. The ion source temperature maintained at 200 °C, while the GC-MS interface (transfer line) temperature at 280°c. The temperatures of the split injector and detector was set at 250 and 230 ℃, respectively. The scanning covered a m/z range of 50 to 550 at a speed of 1666 Hz. The identity of components was then determined by comparing their mass spectra with those in the NIST mass spectral collection.

The relative peak percentage of each identified compound was expressed as;

$$\text{Relative peak percentage}\ (\%) = \frac{Individual\ Peak\ Abundance}{Total\ Peak\ Abundance} \times 100$$

2.5 Antibacterial activity of the crude extracts

The agar well disk diffusion technique by [14] was used to determine the antibacterial and antifungal activity of the crude extracts of *C. aculeatus.* The tests were conducted in the Department of Microbiology laboratory, School of Medicine, Masinde Muliro University of Science and Technology.

The test microorganisms; *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Aspergillus niger* ATCC 16404, and *Aspergillus flavus* ATCC 204304 were a kind donation from the Department of Medical Microbiology, University of Nairobi, and all biosafety protocols were observed during the exchange of the microorganisms. These microorganisms were freshly cultured in the laboratory under aseptic conditions to ensure purity and viability prior to antimicrobial testing.

Inoculum standardization was carried out to ensure uniform microbial density across all tests. For bacterial isolates, a fresh suspension was prepared and then adjusted to match the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL. For fungal isolates, spore suspensions were prepared from actively growing cultures and standardized using hemocytometric counting or turbidity adjustment to obtain a

uniform inoculum concentration. This standardization was done to ensure reproducibility and comparability of results during antimicrobial screening.

By use of a sterile cotton swab, cultures of test organisms were made on Mueller-Hinton agar (for bacteria) and potato dextrose agar (for fungi) plates. Wells of 6.00 mm diameter were drilled into the agar by use of sterile well cutter. The crude extracts were prepared at a concentration of 100 mg/mL, reconstituted in 5% DMSO [15]. Into the wells, 30µL of the individual reconstituted crude extracts were added and allowed to diffuse for 2 h. The plates were then incubated at 37 °C (for bacteria) and 25 °C (for fungi) for 24 h. The assay was done in triplicate. The efficacy of the phytochemicals was determined by measuring the diameter for zones of inhibition in millimeters, with values expressed, as mean ± S.D. Standard positive controls were included for comparison following CLSI guidelines: Ciprofloxacin 5 µg/mL for Gram-negative bacteria, Vancomycin 30 µg/mL for Gram-positive bacteria, and Ketoconazole 10 µg/mL and Voriconazole 1 µg/mL for fungal strains. A volume of 30 µL of each positive control solution was applied per well. 5% DMSO served as the negative control.

2.6 Statistical data analysis

All experimental data obtained from antimicrobial assay were expressed as the mean ± standard deviation, SD of triplicate experiments. The experimental unit was defined as an individual culture well. Statistical significance among multiple groups was determined using the Kruskal–Wallis test, followed by Dunn's post-hoc multiple-comparison test to locate specific differences. All statistical computations were generated using SPSS version 29.0. Differences were considered statistically significant at a threshold of $p < 0.05$.

2.7 Limitations of the study

This study provides an initial evaluation of the chemical profile and biological activities of *Cucumis aculeatus* extracts.

The authors were challenged by limited institutional and regional constraints regarding high-performance analytical instrumentation, and the subsequent mechanical failure of the mass spectrometer's electron multiplier detector at the International Livestock Research Institute (ILRI) facility during optimization trials. Consequently, metabolite characterization was restricted to the untargeted profiling of inherently volatile and semi-volatile constituents via direct GC-MS analysis without prior chemical modification.

Furthermore, the biological activities of single-dose crude extracts were evaluated under in vitro conditions. Additionally, the bioassay-guided fractionation, isolation and characterizing the specific constituents responsible for the observed antimicrobial activity as well as assessing efficacy and safety in in vivo models is beyond the scope of this study.

3 Results and discussions

3.1 Extraction yield

The superior crude extract yield (Table 1) achieved with methanol is attributed to its high dielectric capacity to solubilize polar phyto-constituents including phenols, tannins, and glycosylated triterpenoids (cucurbitacin) present in *Cucumis aculeatus*. Concurrently,

Table 1 Percentage yield for macerated *Cucumis aculeatus* in solvents

Extract Yield (%)	Fresh fruit (500 g)	Dry fruit (500 g)	Dry leaves (500 g)
100% Hexane (1 L)	0.72	1.41	1.68
1:1 DCM/MeOH (1 L)	1.17	3.13	4.38
100% MeOH (1 L)	1.43	3.55	4.74

the intermediate-polarity binary system (1:1 DCM/methanol, v/v) optimized extraction selectivity for mid-polar amphiphilic compounds, whereas the minimal yield from 100% hexane confirms a scarce distribution of non-polar components like hydrocarbons and fatty acids in the plant matrix. Across all solvent systems, dry leaves systematically out yielded fruits, establishing foliar tissues as a more concentrated, metabolically rich repository for target secondary metabolites. Furthermore, the elevated yields observed in dried matrices relative to fresh tissues underscore that post-harvest dehydration successfully concentrates intracellular solutes while eliminating moisture that would otherwise co-dilute the organic solvent and impede matrix penetration.

3.2 GC-MS chemical profile of *Cucumis aculeatus* crude extracts

This study presents the first document report on GC-MS profiling of volatile and semi-volatile phytochemicals from fresh fruit, dry fruit and dry leaves of *C. aculeatus* extracted using hexane, 1:1 dichloromethane/methanol and 100% methanol. Additionally, the in vitro antibacterial and antifungal activities of the extracts are herein reported. This study therefore provides a preliminary reference for further phytochemical and pharmacological investigation on this species, as a source of plant-based antimicrobial agents.

GC-MS was employed to characterize the volatile and semi-volatile constituents of the extracts. Although *Cucumis* species are known to contain non-volatile phytochemicals such as phenolics, flavonoids, and cucurbitacin, these compounds are generally not amenable to GC-MS analysis without derivatization. Therefore, the present GC-MS analysis represents only the volatile fraction of the metabolite profile, while comprehensive characterization of non-volatile metabolites would require further analytical techniques in future.

3.2.1 Chemical profile of hexane extracts from fresh fruit of Cucumis aculeatus

The hexane extract of *C. aculeatus* fresh fruit tentatively yielded a mixture of 6 phytochemicals in different classes including an aromatic ester, saturated and unsaturated hydrocarbons and a long chain fatty alcohol. The most abundant constituent was tentatively identified as 9*E*-nonadecene, accounting for 20.67% of the total number of putatively identified compounds (Table 2; Figs. 2 and 3).

In addition, the analysis revealed the presence of an aromatic ester, benzyl benzoate, which is widely recognized as a fragrance compound. A similar compound was previously reported in *Cucumis melo* [16], thereby providing supportive evidence for its possible occurrence within selected species. Although aromatic esters are not commonly encountered within the *Cucumis* genus, the detection of benzyl benzoate in C. *aculeatus* suggests potential biochemical diversity. This observation reflects the influence of the environment and physiological factors on secondary metabolite biosynthesis.

Table 2 GC–MS chemical profile of hexane extracts from fresh fruit of *Cucumis aculeatus*

Peak No	*R.T.* (min)	Experimental Retention Index (RI)	Similarity index (SI)	Relative Peak percentage (%)	Compound Name	Molecular Formula	Mol. Weight (g/mol)	Class
1	8.125	1852	92	4.33	2,6,10,14-Tetramethylheptadecane)	$C_{21}H_{44}$	296	Branched alkane
2	18.705	2804	91	20.33	*n*-Octacosane ***DFH**	$C_{28}H_{58}$	394	Straight-chain alkane
3	20.535	4395	91	20.00	*n*-Tetratetracontane) ***DLM**	$C_{44}H_{90}$	618	Long-chain alkane
4	21.755	1733	76	15.67	Benzyl benzoate	$C_{14}H_{12}O_2$	212	Aromatic ester
5	21.910	1918	92	20.67	(9*E*)-Nonadecene	$C_{19}H_{38}$	266	Alkene
6	23.145	2252	89	19.00	*n*-Eicosanol ***DLH**	$C_{20}H_{42}O$	298	Fatty alcohol

KEY: *-also present in; DFH-Dry fruit hexane, DLM-Dry leaves methanol, DLH-Dry leaves hexane

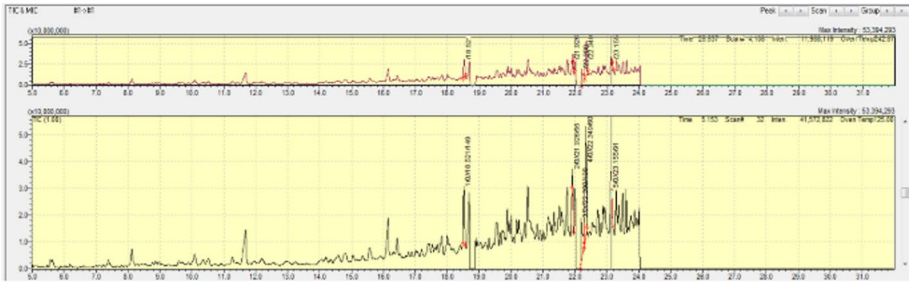


Fig. 2 Total ion chromatogram for hexane extract of *C. aculeatus* fresh fruit

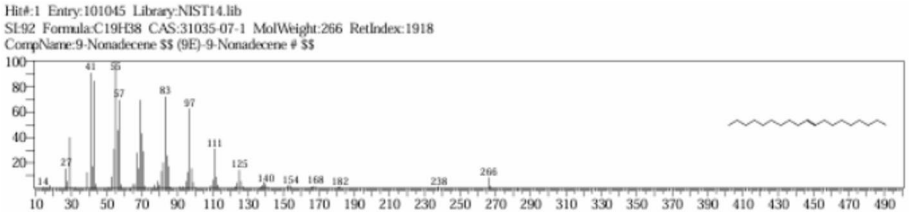


Fig. 3 GC-MS spectrum for the major compound (9*E*)-nonadecene) in hexane extract of *C. aculeatus* fresh fruit

3.2.2 Chemical profile of hexane extracts from dry fruit of Cucumis aculeatus

Alternatively, the hexane extract of *C. aculeatus* dry fruit tentatively yielded 7 phytochemicals belonging to different classes from those harbored by the fresh fruit including; fatty alcohol, alkanes, saturated fatty acid, fatty acid ester and fatty aldehyde (Table 3; Fig. 4). In contrast to the fresh fruit, where a hydrocarbon was the major compound, the dry fruit extract was dominated by a saturated fatty acid, *n*-hexadecanoic acid (26.77% of the total tentatively identified compounds) (Fig. 5).

Among the compounds tentatively identified, *n*-octacosane was common to both the fresh and dried fruit hexane extracts of *C. aculeutus*. However, its relative abundance decreased from 20.33% (Table 2) in the fresh fruit to 5.56% (Table 3) in the dried fruit. The classes and structures of all the other phytochemicals were unique to the method of sample processing. This shows probably that air drying not only removed moisture from the fruit samples as expected, but also slightly altered the phytochemical profile. Such changes could be probably attributed to processes including thermal degradation, which would have led to the loss of volatile compounds, n-eicosanol and benzyl benzoate, that were initially present in the fresh fruit but absent after air drying. The observed differences between the fresh and dried fruit hexane extracts may likely be associated to oxidation, enzymatic transformation, thermal degradation, or changes in metabolite stability during drying, as reported in previous studies [16] However, these mechanisms were not investigated in the present study and therefore remain hypothetical. In this current study, these proposed effects are supported by the presence of more oxygenated compounds; 2-butyl-1-octanol, *n*-hexadecanoic acid, palmitic acid isopropyl ester and *cis*-7,10-hexadecadienal in the dried sample, which were not observed in the fresh sample. Alternatively, the enzymatic transformations may have led to these changes. For instance, reduction of the double bonds followed by oxidation of the aldehydic group of hexadecadienal would lead to *n*-hexadecanoic acid (Table 3). Similarly, enzymatic esterification of *n*- hexadecanoic acid using isopropyl alcohol would yield palmitic acid isopropyl ester in the dried sample (Table 3).

Table 3 GC–MS chemical profile of hexane extracts from dry fruit of *Cucumis aculeatus*

Peak No.	*R*.T (min)	Experimental RI	Similarity index (SI)	Relative Peak Percentage (%)	Compound Name	Molecular Formula	Mol. Weight (g/mol)	Class
1	11.670	1393	90	3.03	2-Butyl-1-octanol	$C_{12}H_{26}O$	186	Fatty alcohol
2	18.650	2804	93	5.56	*n*-Octacosane ***FFH**	$C_{28}H_{58}$	394	Straight chain alkane
3	20.515	1156	90	4.04	2,3,5,8-Tetramethyl- decane	$C_{14}H_{30}$	198	Branched alkane
4	24.310	1968	87	26.77	*n*-Hexadecanoic acid ***DLH**, **FFDM**, **DFDM**, **DLDM**, **DFM**, **DLM**	$C_{16}H_{32}O_2$	256	Saturated fatty acid
5	24.690	2013	86	25.25	Isopropyl ester)	$C_{19}H_{38}O_2$	298	Fatty acid ester
6	25.510	0	89	10.10	2-Methyltetracosane	$C_{25}H_{52}$	352	Branched Alkane
7	26.045	1816	89	25.25	*Cis*-7,10-Hexadecadienal	$C_{16}H_{28}O$	236	Fatty aldehyde

KEY: *-also present in; FFH-Fresh fruit hexane, DLH-Dry leaves hexane, FFDM-Fresh fruit 1:1 dichloromethane/ methanol, DFDM- Dry fruit dichloromethane/ methanol DLDM-Dry leaves 1:1 dichloromethane/ methanol, DFM-Dry fruit methanol, DLM-Dry leaves methanol

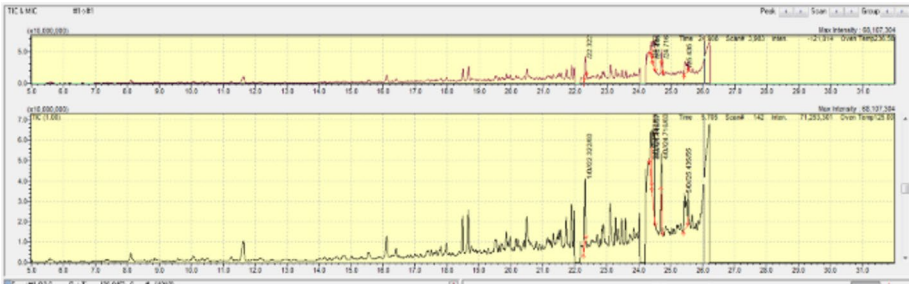


Fig. 4 Total ion chromatogram for hexane extract of *C. aculeatus* dry fruit

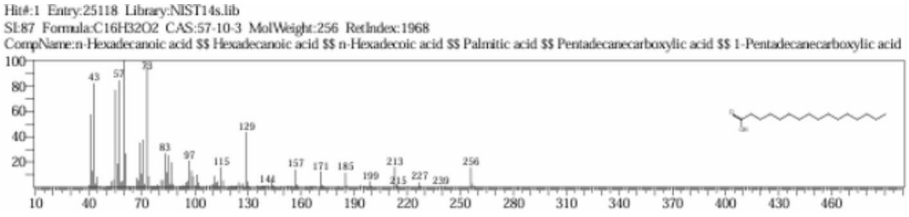


Fig. 5 GC-MS spectrum for the major compound n-hexadecanoic acid in hexane extract of *C. aculeatus* dry fruit

Previous study by [17] reported the occurrence of fatty aldehydes in members of the genus *Cucumis*, emphasizing their key role in the plants' aroma and defense mechanisms. Similarly, volatile analyses of *Cucumis melo* fruits by [18] identified aldehydes among the major classes of compounds detected, further underscoring their significance in defining the characteristic scent and flavor profile of the fruit.

3.2.3 Chemical profile of 1:1 DCM: MeOH extracts from fresh fruit of Cucumis aculeatus

The 1:1 dichloromethane/methanol (1:1 DCM/MeOH) extract of *C. aculeatus* fresh fruit tentatively yielded a polyunsaturated fatty acid methyl ester, 8,11,14-docosatrienoic acid, methyl ester (27.29%) as the tentative major compound (Table 4; Figs. 6 and 7). Compared to the hexane extract, which tentatively showed 7 compounds, the 1:1 DCM/MeOH tentatively revealed a total of 6 compounds. This decrease in the number of detectable phytochemicals can be attributed to differences in solvent polarity and GC-MS detection limitations. Hexane, being non-polar, preferentially extracted volatile compounds, that are readily detectable by GC-MS. In contrast, the 1:1 DCM/MeOH solvent mixture extracted mid polar compounds, primarily fatty acid and their derivatives, (Table 4; Fig. 6). Notably, none of these compounds were detected in the hexane extract, thus supporting the need for varying the solvent for extraction to maximize the diversity of phytochemicals extracted from their sources.

3.2.4 Chemical profile of 1:1 DCM: MeOH extracts from dry fruit of Cucumis aculeatus

The 1:1 DCM/MeOH extracts of *C. aculeatus* dry fruit tentatively yielded a total of 6 fatty acids and their derivatives alongside one alkene. The most abundant compound was tentatively identified as 8,11,14-docosatrienoic acid, methyl ester (27.65%) (Table 5; Figs. 8 and 9). When 1:1 DCM/MeOH solvent mixture was used for extraction, drying of the sample seemed to have had minimal effect on the phytochemical composition. This is because the classes of the compounds were retained and approximately 50%

Table 4 GC–MS chemical profile of 1:1 dichloromethane/methanol extracts from fresh fruit of *Cucumis aculeatus*

Peak No.	*R*.T. (min)	Experimental RI	Similarity index (SI)	Relative Peak Percentage (%)	Compound Name	Molecular Formula	Mol. Weight (g/mol)	Class
1	22.820	1869	80	2.16	Pentadecanoic acid ***DFDM**, **FFM**, **DFM**	$C_{15}H_{30}O_2$	242	Straight chain fatty acid
2	23.585	1878	89	11.26	Hexadecanoic acid, methyl ester ***DFDM**	$C_{17}H_{34}O_2$	270	Fatty acid methyl ester
3	24.365	1968	88	26.84	*n*-Hexadecanoic acid ***DFH**, **DLH**, **DFDM**, **DLDM**, **DLM**	$C_{16}H_{32}O_2$	256	Saturated fatty acid
4	25.530	2499	88	27.29	8,11,14-Docosatrienoic acid, methyl ester ***DFDM**	$C_{23}H_{40}O_2$	348	Poly-unsaturated fatty acid methyl ester
5	26.025	1817	91	5.19	1,*E*-11,*Z*-13-Octadecatriene	$C_{18}H_{32}$	248	Unsaturated hydrocarbon
6	26.275	2183	90	27.25	9,12-Octadecadienoic acid ***DFM**	$C_{18}H_{32}O_2$	280	Polyunsaturated fatty acid

KEY: *-also present in; DFDM-Dry fruit 1:1 dichloromethane/ methanol, DFM-Dry fruit methanol, DLM-Dry leaves methanol, DLDM-Dry leaves 1:1 dichloromethane/ methanol, DFM-Dry fruit methanol, FFM-Fresh fruit methanol

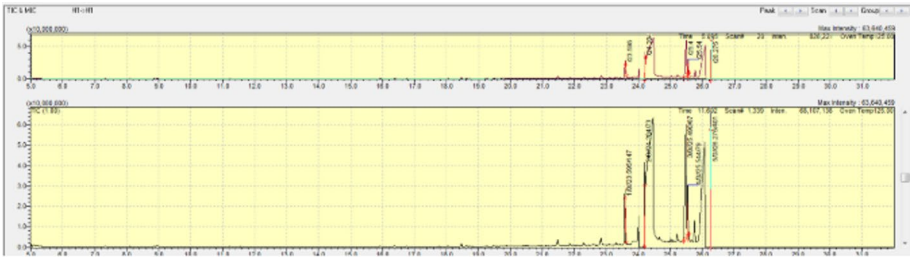


Fig. 6 Total ion chromatogram for 1:1 DCM-MeOH extract of *C. aculeatus* fresh fruit

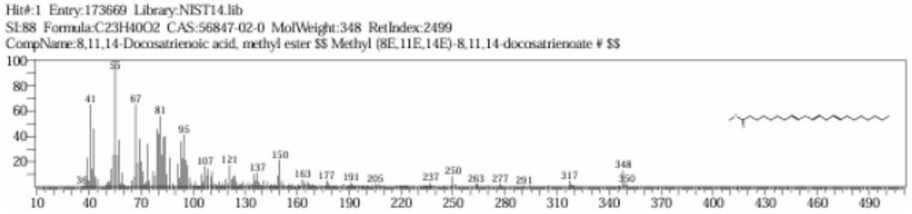


Fig. 7 GC-MS spectrum for the major compound 8,11,14-docosatrienoic acid, methyl ester in 1:1 DCM-MeOH extract of *C. aculeatus* fresh fruit

chemical structure similarity was observed in the extracts from fresh and dried fruit of *C. aculeatus*.

Complementary to these findings [19], provided a comprehensive fatty acid profile of *Cucumis melo*, highlighting a notably high content of linoleic acid. These studies highlight the predominance of polyunsaturated fatty acids in *Cucumis* genus. Moreover, fatty acid methyl esters have been reported primarily in *Cucumis* genus seeds, where hexadecenoic acid represents a principal component of the seed oil [18].

3.2.5 Chemical profile of MeOH extracts from fresh fruit of Cucumis aculeatus

Methanol extraction from the fresh fruits of *Cucumis aculeatus* tentatively yielded only two fatty acid esters, methyl 9-*cis*,11-*trans*-octadecadienoate (76.19%) and pentadecanoic acid, methyl ester (23.81%) (Table 6; Figs. 10 and 11). Compound pentadecanoic acid, methyl ester had been detected in the 1:1 DCM: MeOH extract albeit at a lower percentage of 2.16% (Table 4), therefore indicating that methanol was a more appropriate solvent for its extraction. The limited number of compounds putatively identified in the methanol extract may be attributed to the polarity of the solvent. The extracted compounds were likely to be too polar to be effectively detected and identified using GC-MS without prior derivatization. Consequently, the reduced number of compounds tentatively identified in methanol extracts does not necessarily indicate lower phytochemical diversity but rather highlights analytical limitations of the GC-MS technique when applied to highly polar compounds [20].

3.2.6 Chemical profile of MeOH extracts from dry fruit of Cucumis aculeatus

The methanol extract of *C. aculeatus* dried fruit tentatively yielded 6 compounds (Table 7; Fig. 12) none of which were unique, as they had been earlier detected in the hexane, DCM: MeOH or MeOH extracts of either the fresh of dry extracts. Among the re-extracted compounds, methyl 9-*cis*,11-*trans*-octadecadienoate was the most tentatively abundant (32.63%) (Fig. 13) in the methanolic extract of dry fruit. This observation

Table 5 GC–MS chemical profile of 1:1 dichloromethane/methanol extracts from dry fruit of *Cucumis aculeatus*

Peak No.	*R*.T (min)	Experimental RI	Similarity index (SI)	Relative Peak Percentage (%)	Compound Name	Molecular Formula	Mol. Weight (g/mol)	Class
1	21.505	1869	86	2.65	Pentadecanoic acid ***FFDM**, **FFM**, **DFM**	$C_{15}H_{30}O_2$	242	Fatty acid
2	23.605	1878	89	16.67	Hexadecanoic acid, methyl ester ***FFDM**	$C_{17}H_{34}O_2$	270	Fatty acid ester
3	23.985	1816	79	5.30	7,11-Hexadecadienal	$C_{16}H_{28}O$	236	Fatty aldehyde
4	24.380	1968	88	26.52	*n*-Hexadecanoic acid ***DFH**, **DLH**, **FFDM**, **DLDM**, **DFM**, **DLM**	$C_{16}H_{32}O_2$	256	Fatty acid
5	25.550	2499	87	27.65	8,11,14-Docosatrienoic acid, methyl ester ***DFDM**	$C_{23}H_{40}O_2$	348	Fatty acid ester
6	26.045	0	91	21.21	1,8,11-Heptadecatriene ***DFM**	$C_{17}H_{30}$	234	Alkene

KEY: *-also present in; FFDM-Fresh fruit 1:1 dichloromethane/ methanol, FFM-Fresh fruit methanol, DFM-Dry fruit methanol, DFH-dry fruit hexane, DLH-Dry leaves hexane, DLDM-Dry leaves 1:1 dichloromethane/methanol, DLM-Dry leaves methanol, DFDM-Dry fruit 1:1 dichloromethane/ methanol

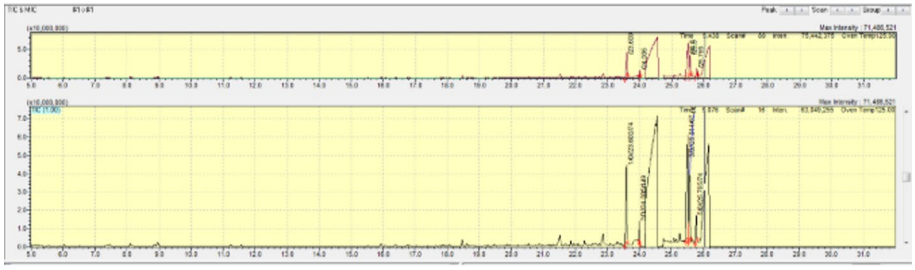


Fig. 8 Total ion chromatogram for 1:1 DCM-MeOH extract of *C. aculeatus* dry fruit

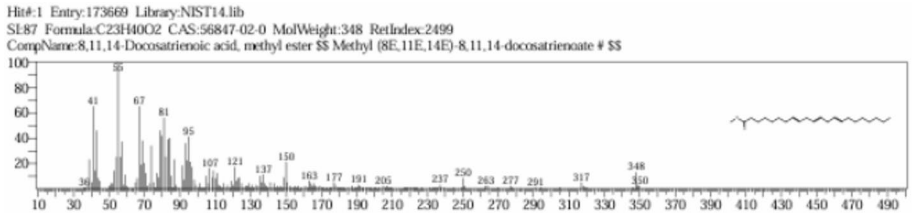


Fig. 9 GC-MS spectrum for the major compound 8,11,14-docosatrienoic acid, methyl ester in 1:1 DCM-MeOH extract of *C. aculeatus* dry fruit

Table 6 GC–MS chemical profile of methanol extracts from fresh fruit of *Cucumis aculeatus*

Peak No.	*R*.T (min)	Experimental RI	Similarity index (SI)	Relative Peak Percentage (%)	Compound Name	Molecular Formula	Mol. Weight (g/mol)	Class
1	23.585	1814	89	23.81	Pentadecanoic acid, methyl ester ***FFDM**, **DFDM**, **DFM**	$C_{17}H_{34}O_2$	270	Fatty acid methyl ester
2	25.435	2093	91	76.19	Methyl 9-cis,11-trans-octadecadienoate ***DLH**, **DFM**	$C_{19}H_{34}O_2$	294	Fatty acid ester

KEY: *-also present in; FFDM-Fresh fruit 1:1 dichloromethane/ methanol, DFDM-Dry fruit 1:1 dichloromethane/ methanol, DLH-Dry leaves hexane, DFM-Dry fruit methanol



Fig. 10 Total ion chromatogram for MeOH extract of *C. aculeatus* fresh fruit

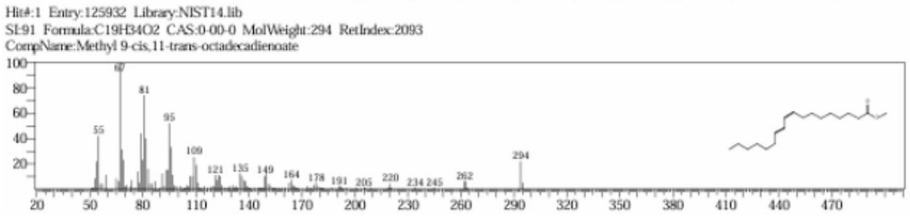


Fig. 11 GC-MS spectrum for the major compound methyl 9-cis,11-trans-octadecadienoate in MeOH extract of *C. aculeatus* fresh fruit

suggests that methanol solvent exhaustively extracted the already detected mid polar phytochemicals and possibly other polar compounds that would not be tentatively identified by the GC-MS due to analytical limitations.

3.2.7 GC-MS chemical profile of extracts from dry leaves of Cucumis aculeatus

A total of 7 compounds (Table 8; Figs. 14 and 15) were tentatively identified in hexane extract, 5 compounds (Table 9; Fig. 16) in 1:1 dichloromethane/methanol extract and 5 compounds (Table 10; Figs. 17 and 18) in methanol extract of the dry leaves of *Cucumis aculeatus* extracts.

The hexane extract of *C. aculeatus* dry leaves tentatively yielded diverse classes of phytochemicals including; alkyne, fatty acid methyl ester, ketone, unsaturated fatty acid methyl ester, saturated fatty acid and polyunsaturated fatty acid methyl ester, with the major compound tentatively identified as methyl 9-*cis*,11-trans octadecadienoate (44.78%) (Table 8; Fig. 15).

Interestingly, alkynes were among the phytochemicals detected in *C. aculeatus*. Previously, alkynes were reported only in trace amounts within the genus *Cucumis*. For instance [21], documented their presence in *Cucumis melo* and *Cucumis sativus*, noting that such components appear at very low concentrations and are not commonly observed across most *Cucumis* genus. The presence of alkynes in dried leaves of *C. aculeatus* therefore suggests biochemical diversity within the genus, potentially influenced by environmental or physiological factors.

The 1:1 DCM/MeOH extracts from dry leaves of *C. aculeatus* tentatively yielded fatty acid esters, fatty acids, fatty alcohol and poly unsaturated fatty acid, with the major compound putatively identified as cyclopentanetridecanoic acid, methyl ester (37.04%) (Table 9; Fig. 17).

On the other hand, the methanol extracts from dry leaves of *C. aculeatus* tentatively harbored unsaturated fatty acid ester, saturated fatty acid, polyunsaturated fatty acid and a polyunsaturated fatty acid ester. Methyl 7,11,14-eicosatrienoate (Table 10; Figs. 18 and 19) was tentatively identified as the major compound in the extract, accounting for 42.27% of the total tentatively identified compounds.

In summary, the GC-MS analysis of the extracts from *C. aculeatus* tentatively revealed an array of phytochemicals including fatty acids, fatty acid esters, alcohols, and hydrocarbons across different plant parts and solvent fractions. The limited detection of characteristic non-volatile phytochemicals, including phenolics, flavonoids, and cucurbitacins, is attributable to the analytical scope of GC-MS, which is inherently biased toward volatile compounds. Future studies employing LC-MS/MS, HPLC, NMR, IR, UV-VIS are recommended to achieve a more comprehensive characterization of the

Table 7 GC–MS chemical profile of methanol extracts from dry fruit of *Cucumis aculeatus*

Peak No.	*R*.T (min)	Experimental RI	Similarity index (SI)	Relative Peak Percentage (%)	Compound Name	Molecular Formula	Mol. Weight (g/mol)	Class
1	23.585	1814	88	23.16	Pentadecanoic acid ***FFDM**, **DFDM**, **FFM**	$C_{17}H_{34}O_2$	270	Fatty acid ester
2	24.120	1968	87	10.00	*n*-Hexadecanoic acid ***DFFH**, **DLH**, **FFDM**, **DLDM**, **DLM**, **DFDM**	$C_{16}H_{32}O_2$	256	Saturated fatty acid
3	25.490	2093	92	32.63	Methyl 9-*cis*,11-*trans*-octadecadienoate ***DLH**, **FFM**	$C_{19}H_{34}O_2$	294	Unsaturated fatty acid ester
4	25.835	1870	84	20.53	*Z*,*Z*-8,10-Hexadecadien-1-ol ***DLDM**	$C_{16}H_{30}O$	238	Unsaturated fatty alcohol
5	25.950	0	91	7.89	1,8,11-Heptadecatriene, ***DFDM**	$C_{17}H_{30}$	234	Hydrocarbon (terpenoid)
6	26.180	2183	83	5.79	9,12-Octadecadienoic acid ***FFDM**	$C_{18}H_{32}O_2$	280	Polyunsaturated fatty acid

KEY: *-also present in; FFDM-Fresh fruit 1:1 dichloromethane/methanol, DFDM-Dry fruit 1:1 dichloromethane/ methanol, FFM-Fresh fruit methanol, DFH-Dry fruit hexane, DLH-Dry leaves hexane, FFDM-Fresh fruit 1:1 dichloromethane/ methanol, DLDM-Dry leaves 1:1 dichloromethane/ methanol, DLM-Dry leaves methanol

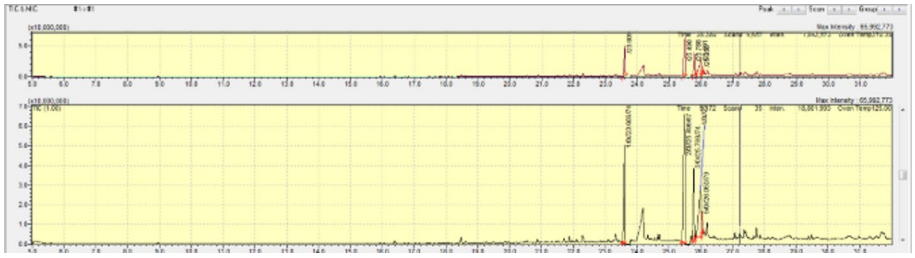


Fig. 12 Total ion chromatogram for MeOH extract of *C. aculeatus* dry fruit

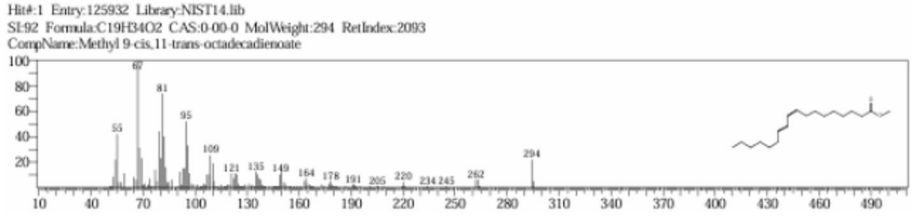


Fig. 13 GC-MS spectrum for the major compound methyl 9-*cis*,11-*trans*-octadecadienoate in MeOH extract of *C. aculeatus* dry fruit

phytochemical composition of *C. aculeatus*. The predominance of fatty acids, esters, and long-chain hydrocarbons observed in the volatile fraction of *Cucumis aculeatus* is consistent with GC–MS studies of several Cucurbitaceae species, where these compounds have been frequently reported among the major volatile constituents. However, differences in the relative abundance and composition of these metabolites may reflect variations in species, plant organ, geographical origin, environmental conditions, developmental stage, and extraction solvent [24].

Fatty acids were consistently detected across different solvent systems, suggesting partial solubility in solvents of intermediate polarity. Specifically, n-hexadecanoic acid was detected in all the three solvent extracts of *C. aculeatus* dried leaf, indicating a broad solubility range. Similarly, the compound n-hexadecanoic acid was detected in all corresponding solvent extracts of the dry fruit, suggesting notable structural stability and consistent chemical behavior across different plant tissues and extraction systems. The recurrence of n-hexadecanoic acid across multiple solvent systems in both the dry fruit and dry leaves indicates its chemical robustness and persistence. This consistence suggests that it's a key phyto-constituent with potential functional and bioactive significance within the plant and warrants further investigation into its pharmacological properties.

The dried fruit tentatively yielded the highest number of compounds (19), followed by dry leaves (17), while the fresh fruit produced the lowest number (14) of compounds based on comparison of their mass spectra with NIST Mass Library and retention index data. This trend suggests that drying of the plant materials is likely to concentrate certain phytochemicals and therefore enhances the extraction of both volatile and semi-volatile compounds.

The current GC-MS analysis predominantly putatively identified known volatile and semi-volatile constituents some that have been reported to possess antimicrobial, among other activities (Table 11). This suggests their individual or synergistic contribution to the observed antibacterial and antifungal effects of the crude extracts of *Cucumis aculeatus* as discussed in the next Sect. 3.3.

Table 8 GC–MS chemical profile of hexane extracts from dry leaves of *Cucumis aculeatus*

Peak No.	*R*.T. (min)	Experimental RI	Similarity index (SI)	Relative Peak Percentage (%)	Compound Name	Molecular Formula	Mol. Weight (g/mol)	Class
1	20.745	1808	93	4.91	1-Octadecyne	$C_{18}H_{34}$	250	Alkyne (long-chain hydrocarbon)
2	22.325	1715	88	7.36	Methyl 13-methyltetradecanoate	$C_{16}H_{32}O_2$	256	Fatty acid methyl ester
3	22.575	1754	85	6.13	2-Pentadecanone,	$C_{18}H_{36}O$	268	Ketone
4	23.335	1886	91	5.52	*Z*- 7-Hexadecenoic acid, methyl ester- ***DLM**	$C_{17}H_{32}O_2$	268	Unsaturated fatty acid methyl ester
5	23.565	1921	85	16.56	Cyclopentaneundecanoic acid, methyl ester	$C_{17}H_{32}O_2$	268	Fatty acid ester (cyclic)
6	24.365	1968	87	14.72	*n*-Hexadecanoic acid ***DFH**, **FFDM**, **DFDM**, **DLDM**, **DFM**, **DLH**	$C_{16}H_{32}O_2$	256	Saturated fatty acid
7	25.510	2093	92	44.78	Methyl 9-*cis*,11-*trans*-octadecadienoate ***FFH**, **DFH**	$C_{19}H_{34}O_2$	294	Polyunsaturated fatty acid methyl ester

KEY: *-also present in; DLM-Dry leaves methanol, DFH-Dry fruit hexane, FFDM-Fresh fruit 1:1 dichloromethane/ methanol, DFDM-Dry fruit 1:1 dichloromethane/ methanol, DLDM-Dry leaves 1:1 dichloromethane/ methanol, DFM-Dry fruit methanol, DLH-Dry leaves hexane, FFH-Fresh fruit hexane, DFH-Dry fruit hexane

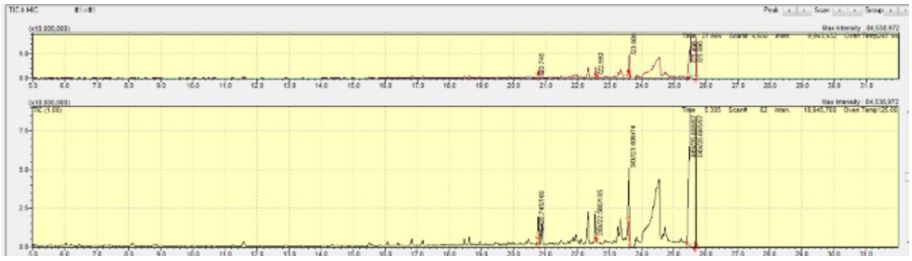


Fig. 14 Total ion chromatogram for hexane extract of *C. aculeatus* dry leaf

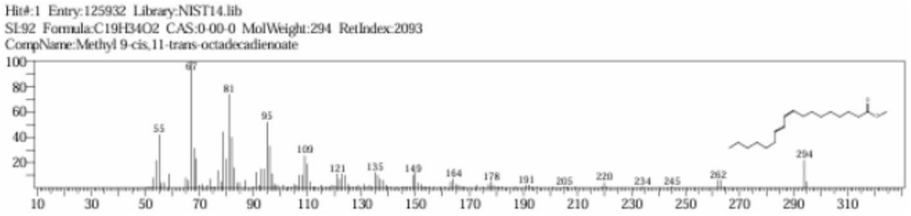


Fig. 15 GC-MS spectrum for the major compound methyl 9-*cis*,11-*trans* octadecadienoate in hexane extract of *C. aculeatus* dry leaf

3.3 Antimicrobial activities of crude extracts from *Cucumis aculeatus*

The extracts from *Cucumis aculeatus* demonstrated a promising growth inhibition of gram negative and gram-positive bacteria, as well as fungi in vitro. The activities were selectively medium to high, as indicated by the zones of inhibition ranging from 9.00 to 17.80 mm, in comparison to the inhibitory effects by the standard drugs used in this study (Fig. 20).

The 1:1 dichloromethane/methanol (DCM/MeOH) and hexane extracts from the fresh fruit of *C. aculeatus* significantly inhibited the growth of the gram-negative *Escherichia coli*, (Fig. 20), with inhibition zones of 14.33 mm and 14.00 mm, respectively. Phytochemical profiling of the bioactive fresh fruit hexane and DCM/MeOH extract revealed a number of known antimicrobial agents (Table 11), whose synergistic action likely led to the observed efficacy against *E.coli*. The remaining extracts moderately inhibited the same microorganism (ZI < 12.00 mm), which was significantly lower than that of ciprofloxacin (ZI = 23.00 mm). These findings correlate well with earlier work by [22], where the hexane root extracts of a related species, *C. prophetarum* showed higher inhibition (ZI = 15.00 mm compared to methanol extract (ZI = 11.60 mm) against *E.coli*.

Similarly, the current study demonstrates an outstanding inhibition of gram positive *S. aureus* by extracts obtained from *C. aculeatus*. The strongest growth inhibition was observed in the dry and fresh fruit hexane extracts (Fig. 20), with mean zones of inhibition of 14.67 mm and 13.67 mm, respectively. These values were closely comparable to vancomycin (ZI = 17.33 mm). All the other plant extracts showed mild and low inhibition against the microorganism with *(*ZI < 12.00 mm). Interestingly, this represents stronger effect towards *S. aureus*, compared to the earlier inhibition of 11.60 mm by hexane root extracts of *C. prophetarum* (24)]. This strong inhibition against gram positive *S. aureus* could be attributed to the additive action of the known antimicrobial non and semi-volatile phytochemicals (Table 11) tentatively identified in the fruits of *C. aculeatus*.

In addition, *C. aculeatus* exhibited promising inhibition against the tested microorganisms. The fresh fruit methanol, dry leaves methanol, dry leaves 1:1 dichloromethane/

Table 9 GC–MS chemical profile of 1:1 dichloromethane/methanol extracts from dry leaves of *Cucumis aculeatus*

Peak No.	*R*.T (min)	Experimental RI	Similarity index (SI)	Relative Peak Percentage (%)	Compound name	Molecular Formula	Mol. Weight (g/mol)	Class
1	23.605	2120	87	37.04	Cyclopentanetridecanoic acid	$C_{19}H_{36}O_2$	296	Fatty acid ester
2	24.060	1968	88	9.26	n-Hexadecanoic acid ***DFH**, **DLH**, **FFDM**, **DFDM**, **DFM**, **DLM**	$C_{16}H_{32}O_2$	256	Fatty acid
3	25.475	2266	87	31.48	Methyl 8-(2-((2-((2-ethylcyclopropyl)methyl) cyclopropyl)methyl)cyclopropyl)octanoate	$C_{22}H_{38}O_2$	334	Fatty acid ester
4	25.700	1870	86	14.81	Z, Z-8,10-Hexadecadien-1-ol ***DFM**	$C_{16}H_{30}O$	238	Fatty alcohol
5	25.930	2390	89	7.41	8,11,14-Eicosatrienoic acid ***DLM**	$C_{20}H_{34}O_2$	306	Polyunsaturated fatty acid

KEY: *-also present in; DFH-Dry fruit hexane, DLH-Dry leaves hexane, FFDM-Fresh fruit 1:1 dichloromethane/ methanol, DFDM-Dry fruit 1:1 dichloromethane/methanol, DFM-Dry fruit methanol, DLM-Dry leaves methanol

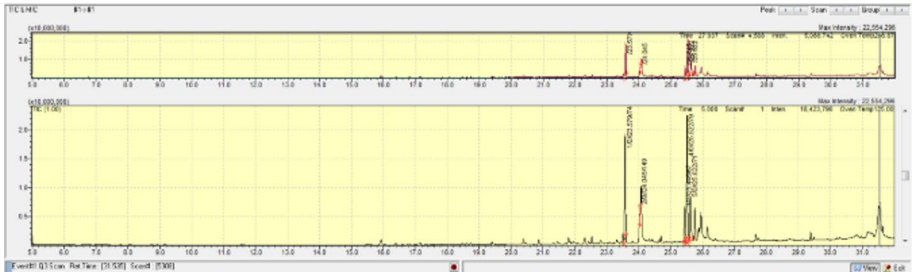


Fig. 16 Total ion chromatogram for 1:1 DCM-MEOH extract of *C. aculeatus* dry leaf

methanol, dry fruit methanol extract and fresh fruit hexane extract showed inhibition zones of 17.80, 17.53, 16.40, 15.40 and 15.27 mm, respectively, against the growth of *Aspergillus niger* (Fig. 21) These values were closely related to those by the standard drug, ketoconazole (ZI = 26.87 mm). The remaining four extracts had moderate activity (ZI < 12.00 mm) against the same microorganism. In comparison, the extracts showed slightly lower inhibition against *A. flavus* (Fig. 21), with the highest inhibition by the dry leaves 1:1 dichloromethane/methanol and fresh fruit methanol extracts with zones of inhibition of 13.73 mm and 13.60 mm, respectively, comparable to 21.00 mm of the standard drug, voriconazole. This study provides the first report of high inhibition of *Aspergillus* species by *C. aculeatus*. Prior to this work, the ethanolic extracts of a sister species *C. anguria* recorded quite low inhibition of 3.70 and 3.30 mm against *A. niger* and *A. flavus*, respectively [25].

From these findings, bioactivity profiling of *C. aculeatus* fruit and leaf extracts revealed a polarity-dependent contrast in antimicrobial efficacy. Polar fractions (1:1 dichloromethane/methanol and methanol) exhibited antifungal properties, whereas non-polar components (hexane) inhibited bacterial pathogens. Although some of the putatively identified mid-polar constituents included known antimicrobial agents, the overall antifungal activity could be as a result of polar phytochemicals that remained un-identified. These findings necessitate targeted downstream identification of the active polar matrix responsible for the antifungal effects.

In summary, several fatty acids and their esters putatively identified in the current study, including *n*-hexadecanoic acid (palmitic acid), have previously been reported to exhibit antimicrobial activity through disruption of microbial membrane integrity, increased membrane permeability, and interference with essential cellular processes (Table 11). *n*-Hexadecanoic acid which was abundant in *C. aculeatus* has previously been reported to possess antimicrobial activity and therefore might have contributed to the activity observed in the crude extracts. Although the present study did not investigate these mechanisms experimentally, these literature reports provide a plausible explanation for the antimicrobial activity observed in the crude extracts [24]. Since the extracts are complex mixtures of phytochemicals, the microbial inhibition may have resulted from individual, synergistic or additive interactions among multiple constituents. Therefore, the results should not be interpreted as evidence that any single detected compound is solely responsible for the observed bioactivity. Future studies involving bioassay-guided fractionation, isolation of active compounds, and evaluation of purified constituents are necessary to identify the specific antimicrobial agents.

Prior to this work [12], reported only mild anti-bacterial activities of leaves and stems extracts of *C. aculeatus* against *E.coli* and *S. aureus*, with inhibition zones of 10.50 mm.

Table 10 GC–MS chemical profile of methanol extracts from dry leaves of *Cucumis aculeatus*

Peak No.	*R*.T (min)	Experimental RI	Similarity index (SI)	Relative Peak Percentage (%)	Compound Name	Molecular Formula	Mol. Weight (g/mol)	Class
1	23.510	1886	89	30.93	7-Hexadecenoic acid, methyl ester (Z) ***DLH**	$C_{17}H_{32}O_2$	268	Unsaturated fatty acid ester
2	24.060	1968	87	7.22	n-Hexadecanoic acid ***DFH**, **DLH**, **FFDM**, **DFDM**, **DLDM**, **DFM**	$C_{16}H_{32}O_2$	256	Saturated fatty acid
3	25.490	2300	90	42.27	Methyl 7,11,14-eicosatrienoate	$C_{21}H_{36}O_2$	320	Polyunsaturated fatty acid ester
4	25.950	2390	89	6.19	8,11,14-Eicosatrienoic acid ***DLDM**	$C_{20}H_{34}O_2$	306	Polyunsaturated fatty acid
5	29.745	4395	94	13.40	n-Tetratetracontane ***FFH**	$C_{44}H_{90}$	618	Long-chain hydrocarbon

KEY: *-also present in; DLH-Dry leaves hexane, DFH-Dry fruit hexane, DFM-Dry fruit methanol, FFH-Fresh fruit hexane, FFDM-Fresh fruit 1:1 dichloromethane/ methanol, DFDM-Dry fruit 1:1 dichloromethane/ methanol, DLDM-Dry leaves 1:1 dichloromethane/ methanol

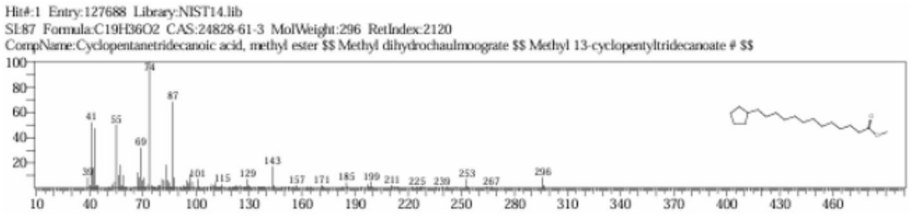


Fig. 17 GC-MS spectrum for the major cyclopentanetridecanoic acid, methyl ester in 1:1 DCM/MeOH extract of *C. aculeatus* dry leaf

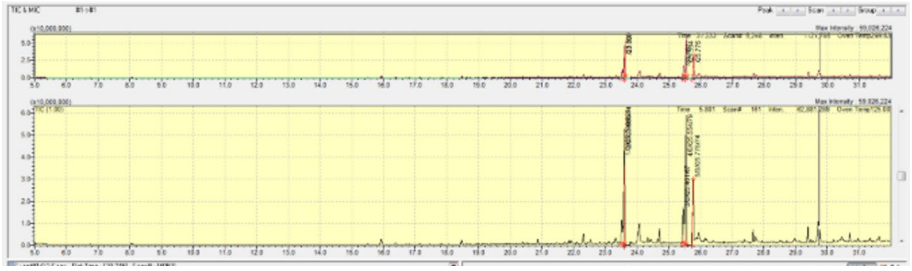


Fig. 18 Total ion chromatogram for MeOH extract of *C. aculeatus* dry leaf

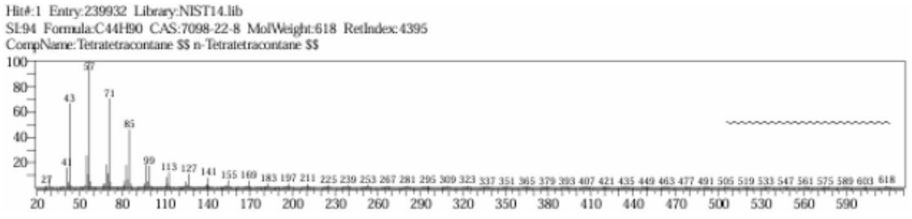


Fig. 19 GC-MS spectrum for the major compound tetratetracontane in MeOH extract of *C. aculeatus* dry leaf

Comparatively, the current study recorded higher inhibition zones of 14.33 mm against *E. coli* and 14.67 mm against *S. aureus.* The variation in plant parts, post-harvest procedure and solvents of extraction employed in the current study could have yielded unique phytochemicals with enhanced microbial inhibition. Collectively, these findings provide an initial justification for the traditional use of *C. aculeatus* as an antimicrobial agent. Furthermore, the results highlight its potential as a source for bioactive phytochemicals that warrant further investigation as potential leads for novel antimicrobial agents.

4 Conclusion

This study tentatively identified the phytochemical composition of the fresh fruit, dry fruit and dry leaves of *C. aculeatus*. The major compounds tentatively detected were; 9*E*-nonadecene, 8,11,14-docosatrienoic acid methyl ester and 9-*cis*,11-*trans*-octadecadienoate in hexane, 1:1 DCM/MeOH and MeOH extracts of fresh fruit *C. aculeatus*, respectively. In the dried fruit, *n*-hexadecanoic acid, 8,11,14-docosatrienoic acid and 9-*cis*,11-trans-octadecadienoate were tentatively dominant compounds in hexane, 1:1 DCM/MeOH and MeOH extracts, respectively. For dried leaves, methyl 9-*cis*,11-*trans* octadecadienoate, cyclopentanetridecanoic acid, and tetratetracontane were the tentative major compounds in hexane, 1:1 DCM/MeOH and MeOH extracts, respectively. Fresh fruit 1:1 DCM/MeOH extracts demonstrated the greatest in vitro inhibitory

Table 11 Reported bioactivities of compounds tentatively identified in extracts from *Cucumis aculeatus*

	Compound	Source	Therapeutic action	Reference
1	Heptadecane 2,6,10,14-tetramethyl-(Pristane)	FFH	Antioxidant, antimicrobial	[26]
2	*n*-Octacosane	FFH, DFH	Antimicrobial, anti-inflammatory, mosquito larvicidal	[27]
3	*n*-Tetratetracontane	FFH, DLM	Antioxidant, antimicrobial, anticancer	[28]
4	Benzyl benzoate	FFH	Antimicrobial, anti-inflammatory	[29]
5	9-Nonadecene(9E)-9-Nonadecene	FFH	Antioxidant, antimicrobial, anticancer	[28]
6	1-Eicosanol	FFH, DLH	Anti-inflammatory, antimicrobial, anticancer	[30]
7	2-Butyl-(2-Butyl-1-octanol)	DFH	Anti-inflammatory, antimicrobial	[30]
8	2,3,5,8-tetramethyl decane	DFH	Antimicrobial	[30]
9	n-Hexadecanoic acid	DFH, DLH FFDM, DFDM, DLDM, DFM, DLM	Antimicrobial, antioxidant, anti-inflammatory	[23]
10	2-Methyltetracosane	DFH	Antibacterial, pesticidal	[30]
11	*Z*,*Z*-7,10-Hexadecadienal	DFH	Antimicrobial, anticancer	[30]
12	1-Octadecyne	DLH	Antimicrobial, anticancer	30]
13	Methyl-13-methyl tetradecanoate	DLH	Antimicrobial, antioxidant, anti-inflammatory	[30]
14	2-Pentadecanone,6,10,14-trimethyl-(hexahydrofarnesylacetone)	DLH	Antimicrobial, anti-inflammatory, antioxidant	[30]
15	*Z*- 7-Hexadecenoic acid, methyl ester	DLH, DLM	Antimicrobial, antioxidant, anti-inflammatory	[30]
16	Cyclopentaneneundecanoic acid, methyl ester (methyldihydrohydrocarpate)	DLH	Antimicrobial, antiparasitic	[30]
17	Hexadecenoic acid, methyl ester	FFDM, DFDM	Anti-inflammatory, antimicrobial, antioxidant	[23]
18	1,*E*-11,*Z*-13-Octadecatrine	FFDM	Antioxidant, antimicrobial	[30]
19	Z, Z-9,12-Octadecadienoic acid	FFDM, DFM	Anti-inflammatory, cardioprotective, wound healing	[31]
20	7,11-Hexadecadienal	DFDM	Antimicrobial	[30]
21	Z, Z- 1,8,11-Heptadecatriene	DFDM, DFM	Antioxidant, antimicrobial	[30]
22	Cyclopentanetridecanoic acid, methyl ester	DLDM	Antimicrobial, antiparasitic, anti-inflammatory	[30]

Table 11 (continued)

	Compound	Source	Therapeutic action	Reference
23	Methyl 8-(2-((2-((2-ethylcyclopropyl)methyl)cyclopropyl) methyl)cyclopropyl)octanoate	DLDM	Antimicrobial, anticancer	[30]
24	Z, Z-8,10-hexadeadien-10-ol	DLDM, DFM	Antimicrobial, insecticidal	[30]

Key: FFH-Fresh fruit hexane, DFH-Dry fruit hexane, DLH-Dry leaves hexane, FFDM-Fresh fruit 1:1 dichloromethane/ methanol, DFDM-Dry fruit 1:1 dichloromethane/ methanol, DLDM-Dry leaves 1:1 dichloromethane/ methanol, DFM-Dry fruit methanol, DLM- Dry leaves methanol

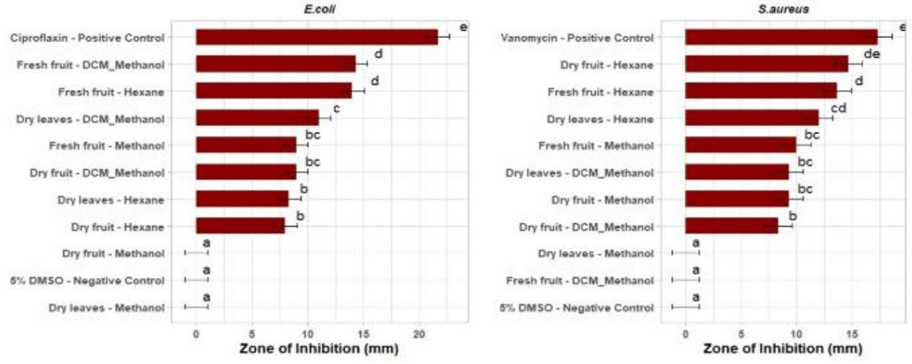


Fig. 20 Antibacterial inhibition activities of plant extracts against *Escherichia coli*, *Staphylococcus aureus.* Groups sharing the same letter are not significantly different (**Kruskal-Walli's test**, **Dunn's post hoc**, $p < 0.05$). Ciprofloxacin and Vancomycin served as positive controls, while DMSO 5% was used as the negative control

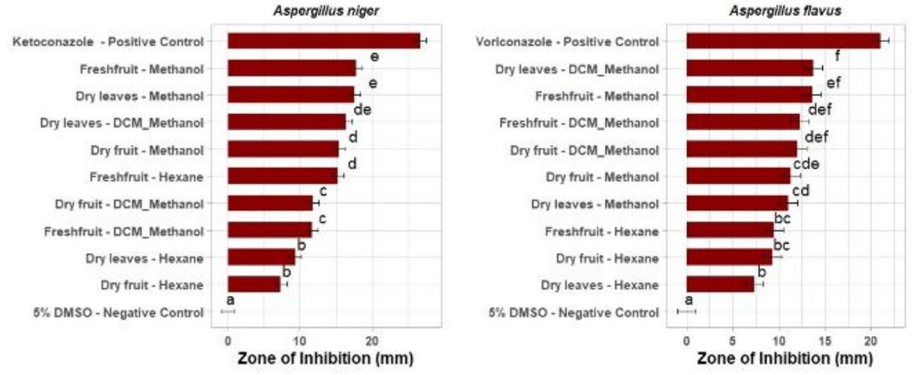


Fig. 21 Antifungal inhibition activities of plant extracts against *Aspergillus niger*, *Aspergillus flavus* Groups sharing the same letter are not significantly different (**Kruskal-Walli's test**, **Dunn's post hoc**, $p < 0.05$). Ketoconazole and Voriconazole served as positive controls, while DMSO 5% was used as the negative control

activity against *E. coli* (14.33 mm). The dry fruit hexane extract demonstrated the greatest inhibitory activity against *S. aureus* (14.67 mm). Fresh fruit methanol and dry leaves 1:1 DCM/MeOH extracts demonstrated the greatest inhibitory activity against *A. niger* and *A. flavus* (17.80 mm and 13.73 mm, respectively). These in vitro antibacterial activities were superior to those reported for closely related plant species. The demonstrated antibacterial and antifungal activities primarily support the traditional use of *C. aculeatus* for managing microbial infections. The extracts demonstrated preliminary in vitro antimicrobial activity against the tested microorganisms. These findings provide a basis for further investigation but should not be interpreted as evidence of clinical efficacy or antimicrobial potency. Additional studies involving MIC/MBC determination,

bioassay-guided isolation, toxicity assessment, and in vivo evaluation are required before therapeutic applications can be considered.

5 Future perspectives

The findings of this study demonstrate that *Cucumis aculeatus* possesses promising in vitro antibacterial and antifungal activity and therefore provides a scientific basis for further pharmacological investigation. However, the development of antimicrobial products from this plant requires additional research. Future studies should focus on bioassay-guided fractionation to isolate and identify the compounds responsible for the observed antimicrobial activity, followed by structural elucidation using complementary analytical techniques such as LC-MS/MS and NMR spectroscopy. The antimicrobial efficacy of the purified compounds should then be evaluated against a broader panel of clinically relevant and multidrug-resistant microorganisms, while investigations into their mechanisms of action should clarify how these compounds inhibit microbial growth.

Subsequent studies should also evaluate cytotoxicity, pharmacokinetic properties, and in vivo efficacy to establish the safety and therapeutic potential of the active compounds. Finally, formulation and stability studies will be necessary to develop suitable dosage forms, such as topical creams, gels, wound dressings, or oral phyto-pharmaceutical preparations, depending on the intended clinical application. Collectively, these investigations will provide the scientific evidence required to translate the preliminary findings of this study into safe, effective, and standardized antimicrobial products.

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Author contributions
F.L.; Lab work, writing original manuscript, G.M.; Conceptualization, writing and reviewing manuscript, supervision, R.K.; Conceptualization, methodology, bench work funds acquisition, supervision, writing and reviewing of manuscript.

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Data availability
All data generated or analysed during this study are included in this manuscript.

Declarations

Ethics approval and consent to participate
Not applicable, as no human study is involved.

Consent for publication
Not applicable, as no human study is involved.

Plant collection guidelines
The collection of the fruits and leaves wild *Cucumis aculeatus* for academic research studies in partial fulfilment for the award of Masters of Science in Chemistry was done in accordance to research rules and guidelines of the school of postgraduate studies of the academic and research institution, Masinde Muliro University of Science and Technology (MMUST). The plant's wild status and significant ethnomedicinal profile prompted the need to characterize its phytochemical composition and evaluate its antimicrobial efficacy. Upon successful review of research proposal, permission was granted by the school of postgraduate studies to collect it for research purposes only, whose outcome would facilitate its propagation and conservation.

Herbarium details
The plant material was identified by Ms. Consolata Shilaho, a taxonomist based at Masinde Muliro University of Science and Technology. The voucher specimen was deposited with accession number (FIL/V/01/2024) at the University Botany Laboratory currently serving as the institution's herbarium for plant samples collected for academic research purposes only.

Competing interests
The authors declare no competing interests.

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