Journal of Natural Products Discovery
https://openjournals.ljmu.ac.uk/JNPD/index
ISSN 2755-1997, 2026, Volume 4, Issue 4, Article 3477

Original article

Phytochemical Profiling, *in-vitro* Antibacterial and Synergistic Assessment of *Harrisonia abyssinica* (Radlk.) Exell and *Dichrostachys cinerea* (L) Wight&Arn. Root Extracts

Tekla Masanja Joseph[1]✉, Sheila Maregesi[1], Adelina Thomas[1], Tanga Mafuru[1], Ramadhani Nondo[2], Vitus Silago[5], Joseph Sempombe[3], Michael Qwarse [4]

[1]Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy, Catholic University of Health and Allied Sciences, P.O. Box 1464, Mwanza, Tanzania.
[2]Department of Biological and Pre-Clinical Studies, Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, P.O. Box 65001, Dar es Salaam, Tanzania.
[3]Department of Medicinal Chemistry, School of Pharmacy, Muhimbili University of Health and Allied Sciences, P.O. Box 65001, Dar es Salaam, Tanzania.
[4]Department of Natural Products, Development and Formulations, Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, P.O. Box 65001, Dar es Salaam, Tanzania.
[5]Department of Microbiology and immunology, Weill Bugando School of Medicine, Catholic University of Health and Allied Sciences, P.O. Box 1464, Mwanza, Tanzania.

D.O.I. 10.24377/jnpd.article3477
Received 2 February 2026; Accepted 20 February 2026; Published 25 February 2026

ABSTRACT

Introduction: The increasing emergence of antimicrobial resistance necessitates the exploration of medicinal plants as alternative sources of novel antibacterial agents. This study investigated the phytochemical composition and in vitro antibacterial activity of *Harrisonia abyssinica* (Radlk.) Exell and *Dichrostachys cinerea* (L) Wight&Arn., two medicinal plants traditionally used in the management of infectious diseases.
Materials and Methods: Crude extracts were subjected to Gas Chromatography–Mass Spectrometry (GC–MS) analysis to identify bioactive constituents, while antibacterial activity was evaluated using the minimum inhibitory concentration (MIC) method against selected pathogenic bacteria. GC–MS analysis revealed the presence of diverse bioactive compounds, including alcohols, fatty acid derivatives, terpenoids, and phenolic compounds, with notable constituents such as 2,3,4-trimethyl-2-pentanol and other oxygenated hydrocarbons detected in *Dichrostachys cinerea*.
Results: Both plant extracts demonstrated measurable antibacterial activity, with MIC values indicating moderate to strong inhibition against tested Gram-positive and Gram-negative bacterial strains. Comparative analysis showed that *Harrisonia abyssinica* exhibited relatively stronger antibacterial effects, which may be attributed to the higher abundance and diversity of antimicrobial phytochemicals**.**
Conclusion: The observed antibacterial activity and their phytochemical constituents reveal the ethnomedicinal use of *Harrisonia abyssinica* and *Dichrostachys cinerea* as promising sources of antibacterial agents for further isolation, characterization, and pre-clinical evaluation of their active compounds.

KEYWORDS: ANTIBACTERIAL ACTIVITY, GC–MS, *HARRISONIA ABYSSINICA*, *DICHROSTACHYS CINEREA*, PHYTOCHEMICAL, ANTIMICROBIAL RESISTANCE.

INTRODUCTION

Bacterial infections continue to pose a major global public health challenge, particularly in low- and middle-income countries where the burden of infectious diseases is impaired by the rapid emergence and spread of antimicrobial resistance (AMR)(Lewnard et al., 2024). The increasing prevalence of resistant bacterial strains has significantly lowered the effectiveness of commonly present and used antibiotics, leading to treatment failures, prolonged illness, increased healthcare costs, and higher mortality rates. This challenge is especially obvious in countries such as Tanzania, where access to newer and more effective antibiotics remains limited and infectious diseases remain a leading cause of morbidity and mortality. Thus, the search for new, effective, affordable, and locally accessible antibacterial agents has become an urgent public health priority (Abdallah et al., 2023).

Medicinal plants have long been used in traditional healthcare practices and have been reported to be a rich source of various bioactive compounds with potential antimicrobial properties. In Africa, and particularly in Tanzania, a substantial proportion of the population relies on plant-based remedies for the treatment of infectious diseases due to their accessibility, affordability, and cultural acceptance(Mapunda and Mramba, 2025). Despite their widespread use, many medicinal plants have not been effectively explored using modern scientific approaches, particularly with respect to their phytochemical composition, antibacterial efficacy, and mechanisms of action. Scientific validation of these traditionally used plants is therefore essential to support their rational use and to identify novel antibacterial compounds that may contribute to future drug development and the mitigation of AMR.

Harrisonia abyssinica and *Dichrostachys cinerea* are medicinal plants traditionally used in the management of various ailments, including infections, inflammatory conditions, and gastrointestinal disorders. Ethnobotanical evidence suggests their use in treating bacterial-related diseases; however, comprehensive experimental evaluation of their antibacterial activity and detailed phytochemical characterization remain limited. The lack of standardized antibacterial testing and compound identification has constrained their acceptance and integration into evidence-based medicine, thereby limiting their potential contribution to addressing antimicrobial resistance (Nyandoro and Munissi, 2024).

Gas Chromatography–Mass Spectrometry (GC–MS) is a widely used analytical technique for the identification and profiling of volatile and semi-volatile phytochemicals in plant extracts(Jha et al.,

2026). When combined with *in vitro* antibacterial assays such as minimum inhibitory concentration (MIC) determination and fractional inhibitory concentration index (FICI) analysis, GC–MS enables correlation between phytochemical composition and observed antibacterial activity(Oseni et al., 2024). This integrated approach provides a scientifically rigorous framework for validating traditional medicinal plants and identifying bioactive compounds responsible for their therapeutic effects.

In the present study, root extracts and fractions of *Harrisonia abyssinica* and *Dichrostachys cinerea* were investigated for their phytochemical composition, *in vitro* antibacterial activity, and potential synergistic interactions against selected Gram-positive and Gram-negative bacterial pathogens. GC–MS analysis revealed the presence of diverse bioactive compounds, including alcohols, fatty acid derivatives, terpenoids, and phenolic compounds. Furthermore, the extracts demonstrated measurable antibacterial activity with varying potency between the two plant species. These findings provide scientific evidence supporting the traditional use of *H. abyssinica* and *D. cinerea* and highlight their potential as sources of novel antibacterial agents in the ongoing fight against antimicrobial resistance.

MATERIALS AND METHODS

MATERIALS

Bacterial Strains

Gram-positive strains used were *Staphylococcus aureus* **(SA),** *Enterococcus faecalis* **(EF1),** and *Enterococcus faecium* **(EF2)***.* Gram-negative strains used were *Escherichia coli* **(EC),** *Klebsiella pneumoniae* **(KP)***.* Two non-pathogenic mycobacteria strains were uses such as *Mycobacterium indicus pranii* **(MIP)** and *Mycobacterium madagascariense* **(MM).** All of the bacterial strains were maintained in nutrients in the biological and pre-clinical and biological laboratory at the Institute of Traditional Medicine (MUHAS).

Other materials and equipment used

Solvent for extracts used DMSO (Dimethyl sulfoxide), distilled water, 70%ethano for disinfections, culture plates, bacterial culture broth (TSB), masks, gloves, plant extracts, aluminum foil, cotton wool, gauze, hot plate, micropipette tips, 8sterile 96-well plate, biosafety cabinet (BSC), Vortex mixer, autoclave, and oven.

Extraction of the plant materials

Root bark samples of Harrisonia abyssinica (T7) and Dichrostachys cinerea (17) were collected, identified, and dried. The dried plant materials were then ground to a coarse powder using a grinding machine to facilitate extraction. The cold maceration method was used with 80% ethanol and 20% distilled water for each sample. A 100 g of powdered material was kept in contact with 80% aqueous ethanol for 48 hours at room temperature with frequent agitation. The extracts were filtered and concentrated using a rotary evaporator at 50°C and reduced pressure to ensure that

labile constituents were not retained (fig 2). Freeze-drying was used to remove water content, and dry extracts were kept at -20°C to maintain the potency of the phytochemicals for biological activity (Fig. **2)**.

Fractions Preparation

The hydroethanolic extracts were first fractionated into fractions of different polarities by completely moistening them with an adequate volume of water, then transferring them into a separating funnel. 200 mL of dichloromethane was added, the mixture was shaken and allowed to settle, and the dichloromethane layer was removed from the bottom of the separating funnel because it is heavier than water. The dichloromethane layer was transferred into a clean container to obtain a fraction. An equal volume of dichloromethane was added again, the mixture was shaken and separated, and the process was repeated thoroughly. A similar cycle was performed for ethyl acetate; however, it was collected from the top layer because it is less dense than water.

Fig 2: *Extract /Fractions Preparation*

Stock solution of the extracts and fractionations preparation

4mg of each extract and fractions were weighed, dissolved in 250 µl of DMSO, and adjusted to 10mg/ml with 750 µl of broth. The solution was vortex-mixed to obtain a clear stock solution of the extracts and fractions.

Broth and Inoculum Preparation

Broth media for Gram-negative and Gram-positive strains were prepared from Tryptic Soy Broth powder by suspending 30 g in 1 liter of distilled water and sterilizing at 121°C for 15 minutes, then allowed to cool to 45-50°C (autoclaved). On the other hand, the Middlebrooks 7H9 broth base supplemented with glycerol was prepared for growing two non-pathogenic Mycobacterium strains and compared to 0.5 McFarland turbidity in transparent glass vials. Preparation was made by measuring 2.45 g of 7H9 powder, adding 450 mL of distilled water, and 2 mL of glycerol according to the manufacturer's instructions. Autoclaving of the broth suspensions was performed at 121°C for 15 minutes, then allowed to cool to 50°C.

Antibacterial bioassays

The MIC was determined by the broth microdilution method, whereby the concentrations were serially diluted, and the bacterial inoculum was standardized to 0.5 McFarland. The 96 microtiter plates were labelled with the name of the plant extract, blank, negative control (extract-free

solution with 80% DMSO and 20% sterilized distilled water), and positive control (Ciprofloxacin) in the first row, and strains of the organism being tested. About 50 µL of each plant extract/fraction, blank, negative control, and positive control were filled in the first rows of each well of 96-well microtiter plates, respectively. Then, 50 µL of the broth media was pre-loaded in each first row of the well. The mixture of the test extracts, blank, negative control, and positive control in the first row was mixed well, and 50 µL of the mixture was transferred to the second row, and the same volume was transferred to the third row until it reached the eighth row, where the last 50 µL of the mixture was discarded. An addition of 50 µL of bacterial suspension was made to make a total of 100µL in each well of the 96-well microtiter plates. The incubation was done for specific temperature for a period of 24 h.

Indicators preparations and observation of color changes

The iodol-nitro tetrazolium indicator was prepared by. Since we used 8 microplates with a total of 800 wells (96x8=768=800 wells), 20 ml will be enough for all wells. INT used is 2mg/ml; therefore, for 20mls, 40mg of iodol-nitro tetrazolium indicator powder was dissolved in 20mls of warm distilled water for the indication of bacterial growth (Gram-negative and Gram-positive bacteria). Addition of 20µlof indicator using micropipette tips in each well was done after 16hours of incubation at 37℃, followed by incubation for 30 minutes. The color changes were observed from colorless to red /pink color, and the minimum inhibitory concentrations were recorded as indicated in the result and appendix sections. The MIC was recorded as the lowest concentration of each drug extract that completely inhibited bacterial growth, as indicated by no visible turbidity (Parvekar et al., 2020).

Phytochemical profiling

Sample preparation

The ethyl acetate fraction obtained from the crude plant extract was concentrated under reduced pressure using a rotary evaporator and allowed to dry completely. A small portion (approximately 1–2 mg) of the dried fraction was reconstituted in analytical-grade ethyl acetate, filtered through a 0.22 µm membrane filter, and transferred into a GC–MS vial for analysis

GC-MS Analysis

Gas chromatography–mass spectrometry (GC–MS) analysis was performed using a GC–MS system equipped with a capillary column (e.g., HP-5MS or equivalent; 30 m × 0.25 mm internal diameter, 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of approximately 1.0 mL/min (Sani et al., 2020)

The injector temperature was maintained at 250 °C, and samples were injected in split mode (split ratio 10:1). The oven temperature program was set as follows: initial temperature at 60 °C (held for 2 min), increased to 280 °C at a rate of 10 °C/min, and held for 10 min. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV, with a scan range of m/z 40–600(Murphy et al., 2005)

Identification of compounds and Data interpretations

Phytochemical constituents present in the ethyl acetate fraction were identified by comparing their mass spectra with those available in the NIST/EPA/NIH mass spectral library. Identification was

further supported by comparing retention times and fragmentation patterns with published literature. Only compounds with a similarity index of ≥80% were considered reliably identified. The relative abundance of each compound was expressed as a percentage of the total peak area in the chromatogram(Eaton, 2015)

Statistical analysis

Antibacterial activity (MIC and FICI)

Minimum inhibitory concentration (MIC) data for individual plant extracts and their combinations were analyzed to determine antibacterial potency and interaction effects. MIC values were recorded as the lowest concentration of extract or extract combination that completely inhibited visible bacterial growth after incubation and expressed in µg/mL. The MIC values were summarized as the mean, with experiments performed in triplicate. Differences in antibacterial activity between individual extracts and their combinations were assessed descriptively by comparing changes in MIC values.

Graphical representation of MIC reductions and FICI values was used to visualize interaction patterns across tested bacterial strains. The nature of interaction between *Harrisonia abyssinica* and *Dichrostachys cinerea* extracts/fractions was evaluated by the Fractional Inhibitory Concentration (FIC) index that was calculated using the checkerboard microdilution method. The FIC for each extract was determined according to the following equations (Pasrija and Kumari, 2025)

$$\text{FIC}_A = \frac{\text{MIC of extract A in combination}}{\text{MIC of extract A alone}}$$

$$\text{FIC}_B = \frac{\text{MIC of extract B in combination}}{\text{MIC of xtrat B alon}} \quad \text{FI Ind (FICI)} = \text{FIC}_A + \text{FIC}_B$$

A reduction in MIC values in the combined extracts compared to individual extracts was considered indicative of synergistic or additive antibacterial interactions. The FICI values were interpreted using standard criteria: Synergistic effect**:** FICI ≤ 0.5, Additive effect**:** 0.5 < FICI ≤ 1.0, Indifferent effect: 1.0 < FICI ≤ 4.0, Antagonistic effect: FICI > 4.0

Active Principles of Harrisonia abyssinica and Dichrostachys cinerea

On the other side, the relative concentration of each identified phytochemical constituent was expressed as a percentage of the total chromatographic peak area on the GC-MS. Peak-area normalization was used to compare the abundance of compounds in the ethyl acetate fraction. Compounds detected in trace amounts (<1% peak area) were reported as minor constituents. All GC–MS analyses were performed in triplicate, and only consistently detected compounds were included in the final phytochemical profile to ensure analytical reproducibility and reliability(Ouandaogo et al., 2023)

Ethical consideration

The ethical clearance for this study was obtained from the Ethical Review Committee **(Ref.No.DA.282/298/01.C/MUHAS-REC-02-2024-2019).** The permission to collect medicinal

plant materials was sought from the President's Office, the Regional Administration and Local Government of Tanzania (PO-RALG) in Magu district.

RESULTS AND DISCUSSION

Antibacterial activity

The study demonstrated that both ethanolic extracts and ethyl acetate fractions exhibited synergistic antibacterial interactions, particularly against the two non-pathogenic mycobacterium strains (MIP and MM) as per the graphical representation of MIC reductions and FICI values ***(Figure 1)*.** However, ethyl acetate fractions showed stronger and broader synergistic effects, reflected by lower MIC values and consistently lower FICI indices. These findings suggest that bioactive compounds enriched in the ethyl acetate fractions **(*T1EA, T2EA, T3EA*)** play a major role in the observed antibacterial synergy. The data are attached to the ***index 1***

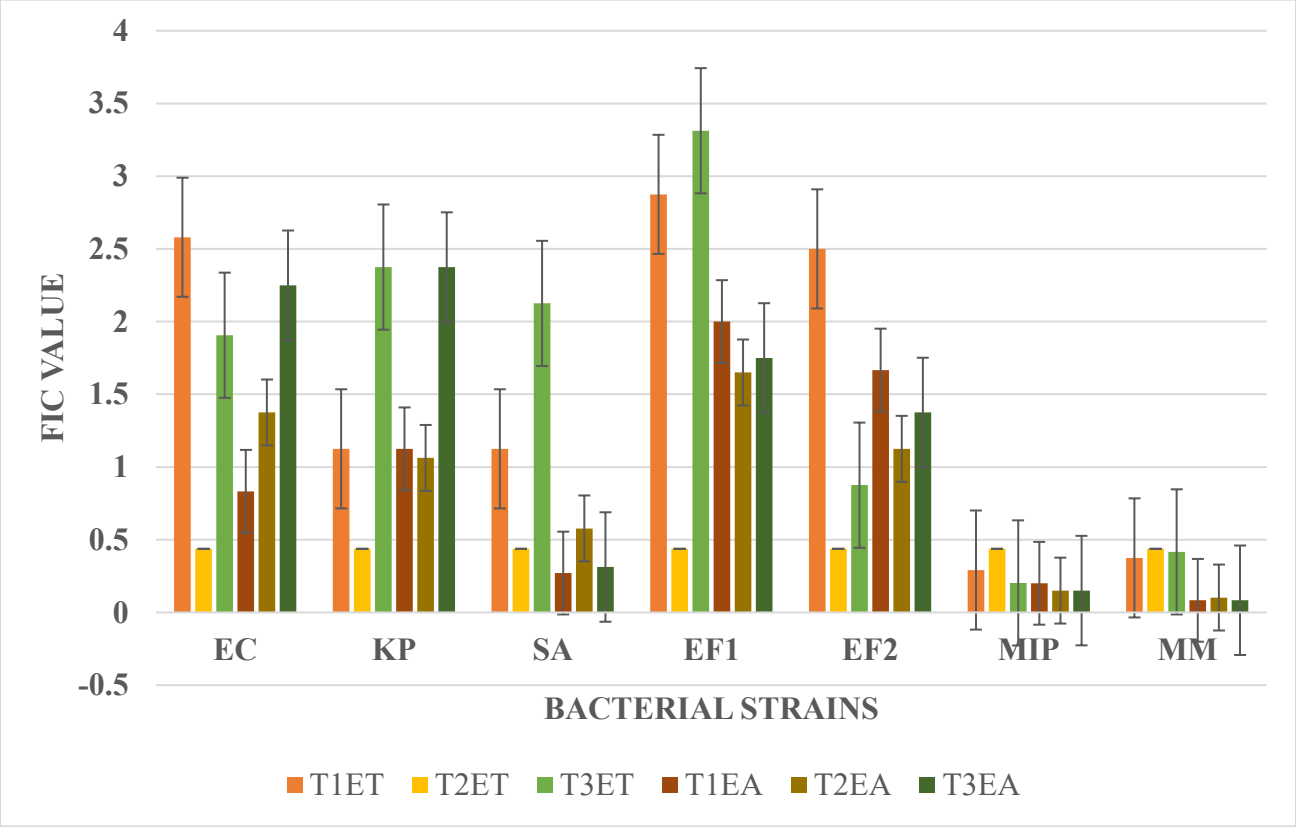

Figure 1: graphical representation of MIC reductions and FICI values whereby: T1ET (Ethanol extracts combination 1:1), T2ET (Ethanol extracts combination 3:1), T3ET (Ethanol extracts combination 1:3), TIEA (Ethyl acetate fractions combination 1:1), T2EA (Ethyl acetate fractions combination 3:1), T3EA (Ethyl acetate fraction combination1:3)

Active Principles of *Harrisonia abyssinica* and *Dichrostachys cinerea*

The GC–MS results revealed the presence of diverse bioactive compounds, including alcohols, fatty acid derivatives, terpenoids, and phenolic compounds in *Harrisonia abyssinica* and *Dichrostachys cinerea* (Table 1 and Table 2).

Table 1: Bioactive principles identified in *Dichrostachys cinerea*

	Compound	Retention Time (Min)	Molecular Formula	MW/ $gmol^{-1}$	AREA %
1	2, 3, 4-trimethy-2-pentanol	5.65	C_8H1_8O	130	2.06
2	Tricyclo[2.2.1.0(2,6)] heptane,1,7-dimethyl-7-(4 methyl-3-pentenyl)-(CAS)	6.9	$C_{15}H_{24}$	204	0.39
3	Phenol,2,4-bis (1,1-dimethylethyl)-(CAS)	7.38	$C_{14}H_{22}O$	206	0.78
4	Phenol,2,5-bis (1,1-dimethylethyl)-(CAS	7.38	C14H22O	206	0.78
5	Phenol, bis(1,1-dimethylethyl)-(CAS)	7.38	$C_{14}H_{22}O$	206	0.78
6	Formic acid,3,7,11-trimethyl-1,6,10-dodecatrien-3-ylester	7.68	C16H26O2	250	0.23
7	4,11-Dimethyl-8-(propan-2-yl)-5,12-dioxatricyclo [9.1.0.04,6] dodecan-7-ol, Ac	7.97	C17H28O4	296	0.38
8	Uvidin C, diacetate	7.97	C19H30O5	338	0.38
10	8-(Hydroxymethyl)-1,5,8-trimethylbicyclo [8.1.0] undec-5-ene-2,9-diol	7.97	$C_{15}H_{24}O_3$	254	0.38
11	Oxireno [9,10] cyclodeca[1,2-b] furan-9(1aH)-one,2,3,6,7,7a,8,10a,10b-octahydro-7-hydroxy-8-(methoxymethyl)-1a,5-dimethyl-, [1Ar (1aR*,4E,7S*,7aR*,8R*,10aS*,10bS*)] -, acetate	8.64	$C_{18}H_{26}O_6$	338	0.23
12	Lactaropallidin	8.9	$C_{15}H_{24}O_3$	252	0.32
13	Desoxyuvidin-B	9.41	$C_{15}H_{24}O_3$	252	0.39
14	5,9b-Dihydroxy-6,6,9a-trimethyl-5,5a,8,9-tetrahydro-3H-benzo[g][2] benzofuran-1,7-dionediacetate	9.94	$C_{19}H_{24}O_7$	364	4.36
15	Pyrene,4-decyl-1,2,3,3a,5a,6,7,8-octahydro- (CAS)	10.04	C26H36	350	0.97
16	Pyrene, 4-decyl-1,2,3,6,7,8-hexahydro- (CAS)	10.04	C26H36	348	0.97
17	3à,4à-Epoxymurolan-9 (11)-en-10-ol	10.69	C15H24O2	236	0.42
18	Hexadecanoic acid, 2,3-dihydroxypropyl ester (CAS)	11.21	C19H38O4	330	0.5
19	n-Hexadecanoic acid (CAS)	11.21	C16H32O2	256	0.5
20	Hexadecanoic acid, ethyl ester (CAS)	11.62	C18H36O2	284	0.5
21	Octadecanoic acid, ethyl ester (CAS)	11.62	C20H40O2	312	0.50

22	2,6,9,12,16-Pentamethylheptadeca-2,6,11,15-tetraene-9-carboxylic acid	11.89	C23H38O2	346	0.42
23	Isochiapin B	12.21	C19H22O6	346	0.22
24	6,8a-Dimethyl-3-methylene-3,3a,4,5,8b,8c-hexahydrooxireno [2',3':2,3] azuleno[4,5-b] furan-2,7-dione	13.78	C15H16O4	260	0.93
25	8-Deoxylactucin	13.78	C15H16O4	260	0.93
26	Acetyloxypart henin	13.78	C17H20O5	304	0.93
27	N-(4-Hydroxyphenyl) retin amide	15.54	C26H33NO2	391	0.38
28	Aissatone	15.93	C20H26O4	330	1.53
29	Ergost-22-en-3-ol, (3á,5à,22E,24R)-(CAS)	16.77	C28H48O	400	1.79
30	Friedelan-3-one	16.77	C30C50O	426	1.79
31	Acetolupuphenone	17.54	C23H32O4	372	12.08
32	Naphtho[2,1-b] furan-4,5-diol, dodecahydro-2-(1-hydroxy-1-methyl-2-propen-1-yl)-3a,6,6,9a-tetramethyl-, triacetate	18.05	C32H54O4	502	0.84
33	D: A-Friedo-2,3-secooleanane-2,3-dioic acid, dimethyl ester, (4R)- (CAS)	18.08	C26H40O7	464	0.84
34	12bS)-2,3-dihydro-12b-methyl-1H-benzo [6,7] phenanthrol[10,1-bc] furan-6,8,11(12bH)-trione	18.94	C20H1404	318	0.37
35	1,4-Bis (4-trimethylsily l-1,3-butadiynyl) benzene	18.94	C20H22Si2	318	0.37
36	Adlupulone	19.51	C26H3804	414	0.51
37	8,13-epoxy-2-methoxylabd-2-en-1-one	20.00	C21H34O3	334	2.37
38	1H-Cyclopropa [3,4] benz[1,2-e] azulene-4a,5,7b,9,9a(1aH)-pentol,1b,4,5,7a,8,9-hexahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-,9,9a-diacetate,[1aR-(1aà,1bá,4aá,5á,7aà,7bà,8à,9á,9aà)]-	20.27	C24H3408	450	0.68
39	Digoxigenin	20.27	C23H34O5	390	0.68
40	11,12-Di-O-methylrosmanol	20.62	C22H30O5	374	1.93
41	Mycophenolic acid IV	20.62	C22H28O6	388	1.93
42	Pregnan-20-one,5,6-epoxy-3,17-dihydroxy-16-methyl-, (3á,5à,6à,16à)-(CAS)	20.86	C22H3404	362	0.25
43	(7R,8S)-Hierochins B	21.41	C21H24O6	372	0.89
44	Hierochins B	21.41	C21H2406	372	0.89
45	Dihydrocoptisine	21.89	C19H15NO4	321	2.11
46	(4aS,6aS,6bR,9R,10S,11R,12aR)-10,11-Dihydroxy-9-(hydroxymethyl)-	22.23	C42H80O5SI4	776	0.59

	2,2,6a,6b,9,12a-hexamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydropicene-4acarboxylicacid, 4TMS				
47	Cannabigerol	22.23	C21H32O2	316	0.59
48	2a,11-Dihydroxy-2,2,4a,7,10b-pentamethyl-1a,2a,3,4,4a,7,8,10,10a,10b,11,11a-dodecahydro-2H,9H- [1] benzoxireno[3,4-f] pyrano[2,3-b] chromen-9-one, A derivative	22.38	C23H32O7	420	0.55
48	2,7:3,6-Dimethanonaphth[2,3-b] oxirene,3,4,6,9,9-pentachloro-1a,2,2a,3,6,6a,7,7a-octahydro-, (1aà,2á,2aá,3á,6á,6aá,7á,7aà)- (CAS)	22.67	C12H9Cl50	344	0.32
49	6H-Dibenzo[b,d]pyran-1,8-diol, 6a,7,8,9,10,10a-hexahydro-6,6,9-trimethyl-3-pentyl-	22.67	C21H32O3	332	0.32
50	2-(16-Acetoxy-11-hydroxy-4,8,10,14-tetramethyl-3-oxohexadecahydrocyclopenta[a]phenanthren-17-ylidene)-6-methyl-hept-5-enoicacid, methyl ester	23.13	C32H48O6	528	0.34
51	3,4'-Dimethyl-2,3'-dioxobicyclohexyl-6-ene-3,4'-dicarboxylic acid, dimethyl ester	23.13	C18H24O6	336	0.34
52	3'-(2-Hydroxypropan-2-yl)-3a",4",6',7"-tetramethyl-5",6-dioxooctahydro-2H,3"H,4H-dispiro[pyran-3,2'-cyclohexane-1',2"-[7,8a] epoxydifuro [2,3-b:3',4'-e] pyran]-5'-yl acetate, TFA	23.49	C29H37F3O11	618	0.62
53	1-Hydroxy-2-(2,3,4,6-tetra-O-acetyl-beta-d-glucopyranosyl)-9H-xanthene-3,6,7-triyltriacetate	24.74	C33H34O18	718	0.39
54	1-(4,6'-Dihydroxy-2',5',5',8a'-tetramethyl-3',4',4a',5',6,6',7',8,8',8a'-decahydro-2'H,3H-spiro[benzo[2,1-b:3,4-c']difuran-2,1'-naphthalen]-8-yl)propan-2-one, 2TFA	25.11	C30H34F6O7	620	2.48
55	Cholest-5-en-3-ol(3á)-, nonanoate	25.11	C36H6202	526	2.48
54	Stigmast-5-en-3-ol,(3á,24S)- (CAS	25.39	C29H50O	414	0.59
55	Trilinolein	25.39	C57H98O6	878	0.59
56	4a,7a-Epoxy-5H-cyclopenta[a]cyclopropa[f]cycloundecene-2,4,7,10,11-pentol,1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-,	25.69	C28H40O10	536	0.77
57	9,19-Cyclo-25,26-epoxyergostan-3-ol,4,4,14-trimethyl-, acetate	25.69	C33H54O3	498	0.77

58	Cholan-24-oic acid,3,12-dioxo-, methyl ester, (5à)- (CAS)	25.69	C25H38O4	402	0.77
59	Prosta-5,8(12),13-trien-1-oic acid,15-hydroxy-9-oxo-, methyl ester, (5Z,13E,15S)- (CAS)	27.36	C21H32O4	348	0.29
60	4-(3-Hydroxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-pentanal	28.11	C27H44O2	400	0.75
61	9,11-Dehydroergosterol peroxide	28.11	C28H42O3	426	0.75
62	Chol-8-en-24-al,3-hydroxy-4,4,14-trimethyl-	28.11	C27H44O2	400	0.75
63	4a-Hydroxy-2,5,7b,10,10,13a-hexamethyl-14-methylene-1,2,4a,5,8,9,9a,10,13a,13b-decahydro-4H,7H-2,7a-methanofuro[3,4-c] naphtho[2,1-e] oxocine-4,7,11(7bH)-trione, TFAderivative	28.55	C27H31F3O7	524	0.33
64	á-Resorcylic acid,5-(3,7-dimethyl-2,6-octadienyl)-6-pentyl-, ethyl ester, (E)- (CAS)	28.55	C24H36O4	388	0.33
65	18-Norcholest-17(20),24-dien-21-oic acid,16-acetoxy-4,8,14-trimethyl-3,11-dioxo-, methyl ester	29.11	C32H46O6	526	0.3
66	2-(16-Acetoxy-4,8,10,14-tetramethyl-3,11-dioxohexadecahydrocyclopenta[a]phenanthren-17-ylidene)-6-methyl-hept-5-enoic acid, methyl ester	29.11	C32H4606	626	0.3
67	5,6,8-Trimethoxy-7-methyl-2,3-diphenylquinoxaline	29.54	C24H22N203	386	0.97
68	9-Desoxo-9x-hydroxy-7-ketoingol3,8,9,12-tetraacetate	29.89	C28H38O10	534	0.22
69	5-Chloro-3-[(1E,3E)-3,5-dimethylhepta-1,3-dienyl]-7,8a-dihydroxy-1-methoxy-7-methyl-1H-isochromene-6,8-dione	30.24	C20H25ClO6	396	0.43
70	Cholest-5-en-3-ol (3á)-(CAS)	30. 62	C27H46O	386	1.94
71	Cholestan-3-one, (5à)-	31.50	C27H46O	386	0.31
72	Olean-12-en-28-oicacid	31.50	C30H48O2	440	0.31
73	à-D-Glucopyranoside, methyl2,3-bis-O-(trimethylsilyl)-, cyclicphenylboronate	31.50	C19H33BO6Si2	424	0.31
74	Toxin T2 tetrol, 4TMS	32.01	C27H5406Si4	586	0.30
75	8-[2-(3,4-Dihydroxy-2,5-dimethoxyoxolan-3-yl) ethyl]-4a,7,8-trimethylspiro[2,3,5,6,7,8a-hexahydro-	32.67	C31H62O8Si3	646	0.30

	1H-naphthalene-4,2'-oxirane]-2,3-diol, 3TMS				
76	Methyl nomilinate(isomer 2)	33.03	C29H380O10	546	0.54
77	R1-Barrigenol	33.77	C30H5006	506	0.53
78	17-Acetyl-12-hydroxy-10,13-dimethylhexadecahydro-3H-cyclopenta[a]phenanthren-3-one, TMS	34.61	C24H40O3Si	404	0.29
79	Benzenehexaethanol,hexaacetate	34.61	C30H42O12	594	0.29
80	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	34.78	C28H40O10	536	0.40
81	Marinobufagin	36.14	C24H32O5	400	0.29
82	Stigmasterol	36.78	C29H48O	412	0.48
83	á-Sitosterol	37.98	C29H50O	414	0.83
84	ç-Sitosterol	37.98	C29H50	414	0.83
85	Noroxymorphone	39.76	C16H17NO4	287	0.25
86	1-(2-Hydroxypropan-2-yl)-3a-methyl-6,10-dimethylidene-2,3,4,5,7,8,9,11,12,12a-decahydro-1H-cyclopenta [11] annulene-5,9-diol	41.81	C20H43O3	322	1.53
87	5,16,20-Pregnatriene-3beta,20-diol diacetate	41.81	C25H34O4	398	1.53
88	5-fluoro PB-223-carboxyindolemetabolite-d5	42.01	C14H11D5FNO2	254	0.38
89	W-18	42.01	C19H20ClN3O4S	421	0.38
90	Stanozolol	42.93	C21H32N2O	440	0.73
91	Betulinaldehyde	43.43	C30H4802	328	0.73
92	R-1 Methanandamide	44.69	C23H39NO2	361	0.83
93	Loperamide	44.69	C29H33ClN2O2	476	0.52
94	Obacunone	50.04	C26H30O7	454	2.65

Table 2. Analysis of bioactive components of T7 *(Harrisonia abyssinica)* by GC-MS

No.	**Compound**	**Retention Time (Min)**	**Molecular Formula**	**MW/ gmol^{-1}**	**% Area**
1	Strychane,1-acetyl-20à-hydroxy-16-methylene- (CAS)	3.80	C21H26N2O2	338	0.59
2	1-(2-Hydroxy-4,6-dimethoxy-3-methylphenyl) ethan-1-one, TMS	4.71	C14H22O4Si	282	0.14
3	4-Amino-5-methyl-2-p yridone, 2TMS	4.71	C12H24N2Osi2	268	0.14
4	R-1 Methanandamide	4.80	C23H39NO2	361	0.16
5	Stanozolol	4.80	C21H32N2O	328	0.16
6	4',6'-Dimethoxy-2'- (trim ethylsilyl) roxychalcone (isomer 2)	6.18	C20H40Si	356	0.23
7	1,1,3,3,5,5,7,7,9,9,11,11-dodecamethylhexasiloxane	7.15	C12H38O5Si6	430	0.35
8	1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-hexasiloxane	7.15	C12H38O5Si6	430	0.35

9	Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	7.15	C16H50O7Si8	578	0.35
10	15-methyltricyclo [6.5.2(13,14).0(7,15)] pentadeca-1,3,5,7,9,11,13-heptene	7.37	C16H14	206	1.95
11	2-Allyl-5-t-butylhydroquinone	7.37	C13H18O2	206	1.95
12	2-tert-Butyl-4-isopropyl-5-methylphenol	7.37	C14H22O	206	1.95
13	3,4-Dihydro-2H-1,5-(3"-t-butyl) benzodiazepine	7.37	C13H18O2	206	1.95
14	2,4-Bis (4-chlorophenyl)-5,6-dihydrobenzo[h]quinazoline	7.50	C24H16ClN2	401	0.21
15	Dimethoxy glycerol docosyl ether	7.50	C27H5605	460	0.21
16	Hexadecamethyl cyclooctasiloxane	8.21	C16H48OSi8	592	0.18
17	1,3-di-iso-propylnaphthalene	9.02	C16H20	212	0.2
18	Loperamide	42.48	C28H33ClN2O2	476	0.15
19	11,13,13,15,15-Hexadecamethyloctasiloxane	64.93	C16H50O7Si8	578	0.22
20	Heptasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamet	64.93	C14H44O6Si7	504	0.22

DISCUSSION

The present study evaluated the antibacterial activity of 80% ethanolic extracts and their corresponding ethyl acetate fractions against a panel of Gram-negative and Gram-positive bacteria, as well as two nonpathogenic Mycobacterium species, using the minimum inhibitory concentration (MIC) assay. The results demonstrate that both crude extracts and ethyl acetate fractions exhibited varying degrees of antibacterial activity, with the ethyl acetate fractions generally showing enhanced potency against several tested organisms.

Antibacterial Activity of 80% Ethanolic Extracts

The 80% ethanolic extracts (T7E, T17E, T1E, T2E, and T3E) exhibited moderate to good antibacterial activity, with MIC values ranging from 0.07 to 2.50 mg/ml. Notably, all ethanolic extracts showed appreciable inhibitory effects against *Mycobacterium* species (MIP and MM), with MIC values as low as 0.07 mg/ml for T3E and 0.09 mg/ml for T1E and T2E. This finding suggests the presence of bioactive constituents with potential antimycobacterial properties, which is consistent with previous reports highlighting the effectiveness of polar to mid-polar phytochemicals in ethanol extracts(Sani et al., 2020)

Against *Staphylococcus aureus* (SA), most ethanolic extracts demonstrated MIC values of 0.38–0.94 mg/ml, indicating moderate activity against Gram-positive bacteria. Activity against Gram-negative organisms such as *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) was comparatively lower, with higher MIC values observed, particularly for T3E (EC: 2.50 mg/ml). This

reduced susceptibility of Gram-negative bacteria may be attributed to the presence of an outer membrane that limits the penetration of phytochemicals(Mossie et al., 2025)

Antibacterial activity of Ethyl Acetate fractions

The ethyl acetate fractions (T7EA, T17EA, T1EA, T2EA, and T3EA) exhibited enhanced antibacterial activity compared to their corresponding crude ethanolic extracts, similar to the previous study done on the stem bark of *Albizia adianthifolia* (Tamokou et al., 2012) . MIC values for these fractions ranged from as low as 0.01 mg/ml to 1.88 mg/ml. Remarkably, T1EA, T2EA, and T3EA showed strong activity against *Staphylococcus aureus*, with MIC values between 0.08 and 0.16 mg/ml, indicating a significant enrichment of antibacterial compounds in the ethyl acetate fraction.

Furthermore, all ethyl acetate fractions demonstrated pronounced antimycobacterial activity. The lowest MIC values (0.01–0.02 mg/ml) were observed for T1EA, T2EA, and T3EA against MIP and MM, surpassing even the positive control in some cases. This suggests that fractionation concentrated mid-polar bioactive constituents, such as phenolics, flavonoids, terpenoids, or fatty acid derivatives, which are known for strong antimycobacterial effects(Singarayar et al., 2025).

Comparison between Extracts and fractions

A clear improvement in antibacterial potency was observed following fractionation into ethyl acetate. This enhancement supports the hypothesis that bioactive compounds are more effectively concentrated in the ethyl acetate fraction than in the crude ethanolic extract. The reduction in MIC values after fractionation also indicates the possible removal of antagonistic or inactive constituents present in the crude extracts.

The data indicate that Gram-positive bacteria (*S. aureus*) were more susceptible to both extracts and fractions than Gram-negative bacteria (*E. coli* and *K. pneumoniae*). This pattern aligns with established antimicrobial susceptibility trends and can be explained by structural differences in bacterial cell walls(Mossie et al., 2025). The positive control demonstrated expected inhibitory activity across all tested microorganisms, validating the reliability of the assay. Conversely, the negative control showed no antibacterial activity (MIC >10 mg/ml), confirming that the observed effects were attributable solely to the plant extracts and fractions.

Synergistic effect of extracts and fractions

The synergistic interactions of combined plant extracts and fractions were evaluated using the fractional inhibitory concentration (FIC) index to determine whether the combinations produced synergistic, additive, indifferent, or antagonistic effects against the tested microorganisms. The findings reveal distinct interaction patterns depending on the type of extract, fractionation, and target organism.

Synergism of combined ethanolic extracts

The combined 80% ethanolic extracts (T1E, T2E, and T3E) predominantly exhibited indifferent interactions against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and the EF1 and EF2 strains, as indicated by FIC values mostly greater than 1.0. These results suggest that, for these organisms, the antibacterial effects of the combined crude extracts were neither enhanced nor diminished when used together (Pasrija and Kumari, 2025)

In contrast, strong synergistic interactions were consistently observed against Mycobacterium species (MIP and MM). All combined ethanolic extracts demonstrated FIC values below 0.5, with T3E showing the lowest FIC values (0.20 for MIP and 0.42 for MM). This indicates a pronounced enhancement of antimycobacterial activity when ethanolic extracts were combined, likely due to complementary mechanisms of action among phytochemicals such as phenolics, alkaloids, and terpenoids. However, an additive effect was observed only in T3E against EF2 (FIC = 0.88), suggesting partial enhancement of antibacterial activity without full synergy. Significantly, no antagonistic interactions were detected among the ethanolic extract combinations, indicating that combined use did not compromise antibacterial efficacy, which is similar to studies done in other plants in previous studies(Donkor et al., 2023).

Synergism of combined ethyl fractions

The combined ethyl acetate fractions exhibited a more pronounced and consistent synergistic profile compared to the crude ethanolic extracts. Notably, all tested ethyl acetate combinations (T1EA, T2EA, and T3EA) showed strong synergistic interactions against Mycobacterium species, with exceptionally low FIC values ranging from 0.08 to 0.20. These findings reinforce the enhanced antimycobacterial potential of mid-polar phytochemicals concentrated in the ethyl acetate fractions.

Against *Staphylococcus aureus*, synergistic effects were observed for T1EA (FIC = 0.27) and T3EA (FIC = 0.31), while T2EA showed an additive effect (FIC = 0.58). This suggests that fractionation improved the cooperative antibacterial interactions of bioactive compounds against Gram-positive bacteria, possibly by increasing the relative abundance of active constituents such as flavonoids and phenolic acids, as also demonstrated in the previous studies (Donadio et al., 2021).

For Gram-negative bacteria (*E. coli* and *K. pneumoniae*), most ethyl acetate fraction combinations resulted in indifferent effects, except an additive effect observed for T1EA against *E. coli* (FIC = 0.83). The lack of synergy against these organisms may be attributed to the protective outer membrane characteristic of Gram-negative bacteria, which limits intracellular accumulation of phytochemicals.

Comparison between ethanolic extracts and Ethyl fractions

Overall, the ethyl acetate fractions demonstrated superior synergistic behavior compared to the crude ethanolic extracts. This improvement can be attributed to fractionation, which likely enriched bioactive compounds while reducing inactive or antagonistic constituents. The consistent synergism observed against Mycobacterium species across both extract types highlights the therapeutic relevance of combining plant-derived compounds in antimycobacterial drug discovery. The observed synergistic and additive effects suggest that the combined extracts and fractions may act through multiple antibacterial mechanisms, including disruption of cell wall synthesis, inhibition of enzymatic pathways, and interference with microbial metabolism. Such multitarget effects are particularly valuable in combating drug-resistant pathogens, especially *Mycobacterium* species(Stelitano et al., 2020)

Phytochemical profile

Bioactive constituents of Dichrostachys cinerea and their Antibacterial Relevance

The GC–MS analysis of *Dichrostachys cinerea* revealed a complex phytochemical composition with several compounds known for antimicrobial and membrane-disruptive activities, as observed in the other plant extract and fractions studies (Masevhe, 2013) .Phenolic derivatives such as 2,4- and 2,5-bis(1,1-dimethylethyl) phenols, resorcylic acid esters, and rosmanol derivatives were identified. Phenolic compounds are well documented for their ability to denature bacterial proteins, disrupt cytoplasmic membranes, and interfere with enzymatic activity, which could be the cause of the notable antibacterial effects observed in MIC and synergism assays (Nassarawa et al., 2023)

Fatty acids and their esters, including n-hexadecanoic acid, hexadecanoic acid ethyl ester, and octadecanoic acid ethyl ester, were also detected. These compounds are known to exert antibacterial activity through disruption of lipid bilayers, increased membrane permeability, and inhibition of fatty acid biosynthesis(Krishnaveni et al., 2014).Their presence likely contributed to the moderate activity against Gram-negative bacteria and enhanced effects when used in combination.

Terpenoids and triterpenes such as friedelan-3-one, olean-12-en-28-oic acid, betulinaldehyde, and ergost-22-en-3-ol were prominent. These compounds are frequently associated with antibacterial and antimycobacterial activities due to their ability to integrate into bacterial membranes and affect cell wall integrity(Konuk and Ergüden, 2020).The presence of steroidal compounds such as stigmasterol, β-sitosterol, and cholest-5-en-3-ol further supports the strong activity observed against Gram-positive bacteria and *Mycobacterium* species, which are particularly susceptible to membrane-active agents.

Notably, alkaloid-related and complex oxygenated compounds such as dihydrocoptisine, noroxymorphone, and marinobufagin were identified in low to moderate abundance. These alkaloids are known to inhibit nucleic acid synthesis and interfere with bacterial metabolic pathways, which may contribute synergistically to the overall antibacterial effect despite their lower relative abundance(Yan et al., 2021)

Moreover, the dominance of acetolupuphenone (12.08% area) and obacunone (2.65% area), both of which are reported to possess antimicrobial and anti-inflammatory activities, suggests that these compounds may represent key contributors to the antibacterial potency of *D. cinerea* extracts and fractions.

Bioactive constituents of Harrisonia abyssinica and their Antibacterial Relevance

The GC–MS profile of *Harrisonia abyssinica* (T7) also demonstrated the presence of multiple antibacterial phytochemicals, although with a comparatively less diverse profile than *D. cinerea*. The detection of phenolic compounds such as 2-allyl-5-tert-butylhydroquinone and 2-tert-butyl-4-isopropyl-5-methylphenol is particularly significant, as these compounds are known for strong antioxidant and antibacterial activities mediated through oxidative stress induction and membrane damage(Nassarawa et al., 2023)

Additionally, nitrogen-containing compounds, including strychnane derivatives and benzodiazepine-related structures, may contribute to antibacterial activity through enzyme inhibition /interference with bacterial signaling pathways. The identification of methanandamide and stanozolol-like steroidal structures suggests the presence of bioactive lipophilic compounds that may enhance membrane permeability and facilitate synergistic interactions with other phytochemicals. The siloxane compounds detected were likely artifacts originating from column

bleeding or derivatization processes and are not considered contributors to biological activity. Nevertheless, the presence of aromatic hydrocarbons and substituted naphthalene derivatives supports the moderate antibacterial activity previously observed for *H. abyssinica* extracts(Alemu et al., 2024)

The antibacterial activity observed in both plants appears to result from the combined action of multiple phytochemical classes rather than a single dominant compound. The coexistence of phenolics, terpenoids, fatty acids, sterols, and alkaloids provides a mechanistic basis for the additive and synergistic effects observed in combination studies. This multi-component nature is particularly relevant in overcoming bacterial resistance mechanisms, as compounds may act simultaneously on bacterial membranes, enzymes, and genetic material. Furthermore, the higher antibacterial and synergistic activities observed in ethyl acetate fractions can be explained by the enrichment of mid-polar compounds such as phenolics and terpenoids, which were abundantly detected in the GC–MS profiles(Masila, 2014).

CONCLUSION

This study demonstrated that *Dichrostachys cinerea* and *Harrisonia abyssinica* possess significant antibacterial and antimycobacterial activities, as evidenced by low minimum inhibitory concentration (MIC) values against a range of pathogenic microorganisms. Both the 80% ethanolic extracts and ethyl acetate fractions exhibited inhibitory effects; however, the ethyl acetate fractions consistently showed superior antibacterial potency, indicating effective enrichment of bioactive constituents following fractionation.

Synergism testing revealed that while combined ethanolic extracts generally exhibited indifferent interactions against most Gram-positive and Gram-negative bacteria, they showed strong synergistic effects against *Mycobacterium* species. In contrast, the ethyl acetate fractions demonstrated enhanced synergistic and additive interactions, particularly against *Staphylococcus aureus* and *Mycobacterium* species, highlighting the therapeutic relevance of phytochemical combinations.

GC–MS analysis identified a wide range of bioactive compounds, including phenolics, terpenoids, fatty acids, sterols, alkaloids, and steroidal derivatives. The presence of compounds such as phenolic derivatives, triterpenes, fatty acid esters, sterols (stigmasterol, β-sitosterol), and antimicrobial-related compounds such as obacunone and acetolupuphenone provides a plausible chemical basis for the observed antibacterial and synergistic effects.

Further studies should focus on the isolation and purification of key bioactive compounds responsible for the observed antibacterial and synergistic effects using complementary analytical techniques such as LC–MS/MS and NMR spectroscopy to confirm compound structures and identify non-volatile phytochemicals. Investigations of the antibacterial and synergistic mechanisms of membrane disruption, enzyme inhibition, and molecular target identification are necessary. Moreover, *in vitro* cytotoxicity assays and *in vivo* toxicity studies should be conducted to evaluate the safety profile of the extracts, fractions, and isolated compounds. Given the strong synergistic effects observed, particularly against *Mycobacterium* species, formulation studies exploring optimized phytochemical combinations should be pursued as potential adjunct therapies.

Generally, the findings validate the ethnomedicinal use of *D. cinerea* and *H. abyssinica* in the treatment of infectious diseases and highlight their potential as sources of novel antibacterial and antimycobacterial agents.

Acknowledgements

We express our gratitude to the Magu district community, government leaders, Traditional Health Practitioners (THPs), and Knowledgeable Elders (KE) where we collect the plant materials. We also thank the Catholic University of Health and Allied Sciences (CUHAS), TPHPA, and Muhimbili University of Health and Allied Sciences (MUHAS).

Funding

This study was funded by the Junior researcher's staff grant from the DVC-ARC office, Catholic University of Health and Allied Sciences.

Conflict of interest

The authors have no conflict of interest regarding this article.

Authors contributions

Term	Definition
Conceptualization	Tekla joseph, Michael Qwarse
Methodology	Tekla Joseph, Ramadhani Nondo, Michael Qwarse
Software	Tekla Joseph, Vitus Silago
Validation	Michael, Qwarse, Tekla Joseph, Joseph Sempombe, Ramadhani Nondo, Adelina Thomas
Formal analysis	Tekla Joseph, Vitus Silago
Investigation	Tekla Joseph, Michael Qwarse
Resources	Michael Qwarse, Tekla Joseph
Data curation	Michael Qwarse, Vitus Silago
Writing original draft	Tekla Joseph
Writing -review & editing	Joseph Sempombe, Ramadhan Nondo, Michael Qwarse
Visualization	Tekla Joseph, Vitus Silago, Michal Qwarse
(Supervision	Joseph Sempombe, Ramadhan Nondo, Michael Qwarse
Project administration	Tekla Joseph
Funding acquisition	Tekla Joseph

Supplementary Materials

Table S1: Minimum Inhibitory Concentrations (MIC) of 80% Ethanolic in mg/ml of Single and Combined Extracts (Mean and standard deviations)

No	Sample name	EC	KP	SA	EF1	EF2	MIP	MM
2	T17E	0.5±0	0.75±0.25	0.375±0.1443	0.25±0	1.875±0.625	0.390125 ±0	0.2926±0.1126
3	T1E	1.25±0	0.9375±0.3125	0.46875±0.1805	0.9375±0.3125	1.875±0.626	0.0975 ±0	0.5857±0.2259
4	T2E	0.9375±0.3125	0.9375±0.3126	0.46875±0.1806	0.9375±0.3206	0.9375±0.3206	0.0975 ±0	0.14626±0.0563
5	T3E	2.5±0	1.875±0.625	0.9375±0.3125	0.9375±0.3607	0.9375±0.3206	0.0731±0.0282	0.1950±0
6	Positive C	0.42±0	0.125ug/ml±0	0.42ug/ml±0	0.83±0	0.83±0	0.50±0.50	0.50±0.50
7	Negative C	> 10	>10	>10	>10	>10	>10	>10

Table S2: Minimum Inhibitory Concentrations (MIC) in mg/ml of Single and Combined Ethyl Acetate Fractions

Minimum Inhibitory Concentrations in mg/ml of Single and Combined Ethyl Acetate Fractions						
Sample Name	**EC**	**KP**	**SA**	**EF1**	**EF2**	**MIP**
T7EA	0.625, 0.625	1.25, 0.625	0.15625, 0.15625	0.625, 0.3125	0.3125, 0.625	0.097625, 0.0488
T17EA	1.25, 0.625	0.625, 0,625	2.5, 1.25	0.3125, 0.15625	0.3125, 0.3125	0.0976525, 0.097625
T1EA	0.625, 0.625	2.5, 1.25	0.078125, 0,078125	0.625, 0.625	0.625, 0.625	0.0195, 0.0195
T2EA	1.25, 0.625	2.5, 1.25	0.15625, 0.078	0.625, 0.625	0.3125, 0.625	0.0097, 0.0195
T3EA	2.5, 1.25	1.25, 0.625	0.15625, 0.15625	1.25, 0.625	0.625, 0.3125	0.00488, 0.024
POSITIVE Control	0.42ug/ml	0.125ug/ml	0.42ug/ml	0.83	0.83	0.83
Negative Control	above 10	above 10	above 10	above 10	above 10	above 10

3.2 Synergism Testing

Table S3: Fractional Inhibitory Concentration Index (FIC) of Combined Ethanol Extracts

Sample Test	Parameters	EC	KP	SA	EF1	EF2	MIP	MM
T1E	FIC Value	2.58	1.13	1.13	2.88	2.50	0.29	0.38
	Effect	Indiff	Indiff	Indiff	Indiff	Indiff	Synergy	Synergy
T2E	FIC Value	1.97	1.06	1.06	2.44	1.63	0.31	0.44

	Effect	Indiff	Indiff	Indiff	Indiff	Indiff	Synergy	Synergy
T3E	FIC Value	1.91	2.38	2.13	3.31	0.88	0.20	0.42
	Effect	Indiff	Indiff	Indiff	Indiff	Addict	Synergy	Synergy

Table S4: Fractional Inhibitory Concentration Index (FIC) Combined Ethyl Acetate Fractions

Sample Test	Parameters	EC	KP	SA	EF1	EF2	MIP	MM
T1EA	FIC Value	0.83	1.13	0.27	2.00	1.67	0.20	0.08
	Effect	Addict	Indiff	Synergy	Indiff	Indiff	Synergy	Synergy
T2EA	FIC Value	1.38	1.06	0.58	1.65	1.13	0.15	0.10
	Effect	Indiff	Indiff	Addict	Indiff	Indiff	Synergy	Synergy
T3EA	FIC Value	2.25	2.38	0.31	1.75	1.38	0.15	0.08
	Effect	Indiff	Indiff	Synergy	Indiff	Indiff	Synergy	Synergy

References

Abdallah, E.M., Alhatlani, B.Y., de Paula Menezes, R., Martins, C.H.G., 2023. Back to nature: Medicinal plants as promising sources for antibacterial drugs in the post-antibiotic era. Plants 12, 3077.

Alemu, B., Molla, M.D., Tezera, H., Dekebo, A., Asmamaw, T., 2024. Phytochemical composition and in vitro antioxidant and antimicrobial activities of Bersama abyssinica F. seed extracts. Sci. Rep. 14, 6345.

Donadio, G., Mensitieri, F., Santoro, V., Parisi, V., Bellone, M.L., De Tommasi, N., Izzo, V., Dal Piaz, F., 2021. Interactions with microbial proteins driving the antibacterial activity of flavonoids. Pharmaceutics 13, 660.

Donkor, M.N., Donkor, A.-M., Mosobil, R., 2023. Combination therapy: synergism among three plant extracts against selected pathogens. BMC Res. Notes 16, 83. https://doi.org/10.1186/s13104-023-06354-7

Eaton, A.L., 2015. Isolation and Synthesis of Bioactive Compounds from Plants.

Jha, D.K., Dadwal, V., Bhattarai, S., Balyan, S., Patil, B.S., 2026. Gas chromatography–mass spectrometry-based metabolomics—an effective method for qualitative and quantitative analysis of phytochemicals, in: Phytochemical Analysis by Modern Techniques. Elsevier, pp. 317–349.

Konuk, H.B., Ergüden, B., 2020. Phenolic –OH group is crucial for the antifungal activity of terpenoids via disruption of cell membrane integrity. Folia Microbiol. (Praha) 65, 775–783. https://doi.org/10.1007/s12223-020-00787-4

Krishnaveni, M., Dhanalakshmi, R., Nandhini, N., 2014. GC-MS analysis of phytochemicals, fatty acid profile, antimicrobial activity of Gossypium seeds. Int J Pharm Sci Rev Res 27, 273–276.

Lewnard, J.A., Charani, E., Gleason, A., Hsu, L.Y., Khan, W.A., Karkey, A., Chandler, C.I., Mashe, T., Khan, E.A., Bulabula, A.N., 2024. Burden of bacterial antimicrobial resistance in low-income and middle-income countries avertible by existing interventions: an evidence review and modelling analysis. The Lancet 403, 2439–2454.

Mapunda, P.E., Mramba, R.P., 2025. Medicinal plant use and conservation in Tanzania: perspectives from university of Dodoma students. Discov. Soc. Sci. Health 5, 77. https://doi.org/10.1007/s44155-025-00232-2

Masevhe, N.A., 2013. Isolation and characterization of antifungal compounds from Clerodendron glabrum var glabrum (Verbenaceae) used traditionally to treat candidiasis in Venda, South Africa. University of Pretoria (South Africa).

Masila, V.M., 2014. Phytochemical investigation of harrisonia abyssinica and thespesia garckeana for antiplasmodial and antimicrobial compounds. (PhD Thesis). University of Nairobi.

Mossie, T., Kassa, T., Urge, B., 2025. Evaluation of Antibacterial Activity of *Trichilia dregeana* Sond. (Meliaceae) Methanolic Extract Against Gram-Positive and Gram-Negative Bacteria. Vet. Med. Sci. 11, e70231. https://doi.org/10.1002/vms3.70231

Murphy, R.C., Barkley, R.M., Zemski Berry, K., Hankin, J., Harrison, K., Johnson, C., Krank, J., McAnoy, A., Uhlson, C., Zarini, S., 2005. Electrospray ionization and tandem mass spectrometry of eicosanoids. Anal. Biochem. 346, 1–42.

Nassarawa, S.S., Nayik, G.A., Gupta, S.D., Areche, F.O., Jagdale, Y.D., Ansari, M.J., Hemeg, H.A., AL-Farga, A., Alotaibi, S.S., 2023. Chemical aspects of polyphenol-protein interactions and their antibacterial activity. Crit. Rev. Food Sci. Nutr. 63, 9482–9505. https://doi.org/10.1080/10408398.2022.2067830

Nyandoro, S.S., Munissi, J.J., 2024. Biomedical Potential of Natural Products from Selected Tanzanian Flora: A Review. Tanzan. J. Sci. 50, 789–812.

Oseni, T.E., Adejoh, M.E., Omowaye, O.S., Attah, F., Peter, J., Tennyson, M.A., Oladipe, T.T., Olubiyo, C.K., Oludare, T.T., Odiba, J.C., 2024. GC-MS analysis, qualitative and quantitative phytochemical composition of Boerhavia diffusa (Linn.) leaf extract characterizing its medicinal use. FUDMA J. Sci. 8, 144–151.

Ouandaogo, H.S., Diallo, S., Odari, E., Kinyua, J., 2023. Phytochemical Screening and GC-MS Analysis of Methanolic and Aqueous Extracts of *Ocimum kilimandscharicum* Leaves. ACS Omega 8, 47560–47572. https://doi.org/10.1021/acsomega.3c05554

Pasrija, R., Kumari, D., 2025. Checkerboard Analysis to Calculate the Fractional Inhibitory Concentration (FIC) Index of Pathogenic Fungi for Drug Interaction, in: Protocols for Studying Pathogenic Fungi. Springer Nature Singapore, Singapore, pp. 109–116. https://doi.org/10.1007/978-981-96-7047-5_9

Sani, M.S.A., Bakar, J., Rahman, R.A., Abas, F., 2020. Effects of Coated Capillary Column, Derivatization, and Temperature Programming on the Identification of Carica papaya Seed Extract Composition Using GC/MS Analysis. J. Anal. Test. 4, 23–34. https://doi.org/10.1007/s41664-020-00118-z

Singarayar, M.S., Chandrasekaran, A., Neethirajan, V., Balasundaram, D., Veerasamy, V., Thilagar, S., 2025. Solvent Polarity-Driven Phytochemical Profiling and Antioxidant Evaluation of Indian Persimmon (Diospyros Kaki Thunb.) Fruit Extracts. J Phytol 17, 91–104.

Stelitano, G., Sammartino, J.C., Chiarelli, L.R., 2020. Multitargeting compounds: a promising strategy to overcome multidrug-resistant tuberculosis. Molecules 25, 1239.

Tamokou, J.D.D., Simo Mpetga, D.J., Keilah Lunga, P., Tene, M., Tane, P., Kuiate, J.R., 2012. Antioxidant and antimicrobial activities of ethyl acetate extract, fractions and compounds from stem bark of Albizia adianthifolia (Mimosoideae). BMC Complement. Altern. Med. 12, 99. https://doi.org/10.1186/1472-6882-12-99

Yan, Y., Li, X., Zhang, C., Lv, L., Gao, B., Li, M., 2021. Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. Antibiotics 10, 318.