Egypt. J. Chem. **Vol. 68**, No. 4, pp. 205 - 221 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Antioxidant and Antimicrobial Activities of *Terminalia laxiflora* and *Terminalia myriocarpa* Leaves in Relation to Their GC-MS and UPLC-ESI-MS Metabolite Profiling

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Abstract

Terminalia L. is the second-largest genus of the Combretaceae family. Numerous species of *Terminalia* were identified to possess antioxidant and antimicrobial properties. This study aimed to evaluate the antioxidant and antimicrobial activities of fractions derived from *Terminalia laxiflora* and *Terminalia myriocarpa n*-hexane and hydromethanolic leaf extracts and to analyze the phytoconstituents responsible for these activities. The percentage of the antioxidant activity (AA%) of each test fraction was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. Additionally, the antimicrobial activity of each test fraction was evaluated *in-vitro* by the agar well diffusion method against five microbial strains. The minimum inhibitory concentrations (MICs) of the test fractions were determined using the broth macrodilution method. The constituents of each fraction that showed antioxidant and/or antimicrobial potential were tentatively identified using GC-MS and UPLC-ESI-MS techniques. The results showed that all the tested fractions possess antioxidant and antimicrobial capabilities. Twenty-five and twenty-three compounds were identified in *Terminalia laxiflora* leaf fractions using GC-MS and UPLC-ESI-MS, respectively. Similarly, twenty-nine and twenty-six compounds were identified in *Terminalia myriocarpa* leaf fractions using GC-MS and UPLC-ESI-MS, respectively. Some of these constituents were reported to display antioxidant and antimicrobial properties, which could explain the observed effects of the fractions.

Keywords: *Terminalia laxiflora*; *Terminalia myriocarpa*; antioxidant; antimicrobial; GC-MS; UPLC-ESI-MS.

Introduction

Terminalia L. (Combretaceae) comprises about 250 species of medium to large trees distributed in tropical and subtropical Africa, southern Asia, the Himalayas, Madagascar, and Australia [1]. About thirty native *Terminalia* species are thought to exist in Africa [2]. Natural antioxidants are essential for maintaining human health because they prevent cell damage by oxidative stress [3]. They exert prophylactic effects against a range of ailments, including neurodegenerative diseases, cancer, diabetes, and inflammatory bowel syndrome [3,4]. Many *Terminalia* species were formerly found to possess antioxidant properties [1]. For instance, ellagic acid and terchebulin, isolated from *T. laxiflora* wood, showed a DPPH radical scavenging activity close to that of (+)-catechin [5]. In addition, it was found that the antioxidant properties of *T. myriocarpa* and *T. muelleri* leaf extracts match those of rutin, iron sulphate, and gallic acid [6].

Compounds derived from natural sources are also renowned for their antimicrobial properties against a wide array of pathogens [7]. Several components isolated from *Terminalia* species were discovered to have antimicrobial properties against a range of microbes [1]. For example, terchebulin and flavogallonic acid dilactone, isolated from *T. laxiflora* methanolic wood extract, exhibited antibacterial effects against *Propionibacterium acnes* and *Streptococcus sobrinus*, respectively [5,8]. Also, the extracts of *T. laxiflora* stem bark, stem wood, and roots were reported to possess antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, and *Pseudomonas aeruginosa* [9]. Moreover, the root extract of *T. laxiflora* showed an inhibitory potential against *Mycobacterium smegmatis* [10]. However, another study

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Receive Date: 27 June 2024, Revise Date: 08 August 2024, Accept Date: 26 August 2024
DOI: 10.21608/ejchem.2024.299712.9908

reported that the total methanolic extract of *T. laxiflora* leaves exhibited no antibacterial action against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, or *Pseudomonas aeruginosa* [11].

Previous reports on the antioxidant and antimicrobial potential of different fractions derived from *T. laxiflora* and *T. myriocarpa* leaf extracts are rather rare. The investigation of fractions rather than crude extracts helps to benefit from the improved constituent resolution in these fractions and to establish a correlation between the observed bioactivities of each fraction and its relative qualitative and quantitative composition. This prompted the current assessment of the DPPH scavenging activity of the fractions derived from *T. laxiflora* and *T. myriocarpa* *n*-hexane and hydromethanolic leaf extracts, as well as their antimicrobial potential against the gram-positive bacteria *Bacillus subtilis* (NRRL B-543) and *Staphylococcus aureus* (NRRL B-313), gram-negative bacteria *Escherichia coli* (NRRL B-210) and *Klebsiella pneumonia* (NCIMB 10341), as well as the yeast *Candida albicans* (NRRL Y-477). The study also aimed at the tentative characterization of the constituents accounting for the DPPH scavenging and antimicrobial effects of the investigated fractions by GC-MS and UPLC-ESI-MS techniques.

1. Results and Discussion

1.1. DPPH scavenging activity

The DPPH scavenging assay showed that all the tested *T. laxiflora* and *T. myriocarpa* leaf fractions possess antioxidant capabilities **(Table I and Figure 1)**. Among *T. laxiflora* leaf fractions, the ethyl acetate (LE) and *n*-butanol (LB) fractions exhibited comparable antioxidant effects (Antioxidant activity = 77 ± 0.14 and 76 ± 0.34 %, respectively) to that of L-ascorbic acid (Antioxidant activity = 88 ± 3.68 %). Concerning *T. myriocarpa* leaf fractions, the ethyl acetate (ME) fraction exerted a more potent antioxidant effect (Antioxidant activity = 92 ± 0.08 %) than that of L-ascorbic acid (Antioxidant activity = 88 ± 3.68 %).

Table I: Percentages of antioxidant activity (AA%) of *T. laxiflora* and *T. myriocarpa* leaf fractions estimated by DPPH radical scavenging assay

Sample (100 µg/mL)	Percentage of antioxidant activity (AA%)
LH	8 ± 0.04
MH	11 ± 0.09
LD	21 ± 0.06
MD	80 ± 0.13
LE	77 ± 0.14
ME	92 ± 0.08
LB	76 ± 0.34
MB	84 ± 0.12
L-Ascorbic acid	88 ± 3.68

LH, MH: Unsaponifiable matters of *T. laxiflora* and *T. myriocarpa* leaves, respectively. **LD, MD:** Dichloromethane fractions of *T. laxiflora* and *T. myriocarpa* leaves, respectively. **LE, ME:** Ethyl acetate fractions of *T. laxiflora* and *T. myriocarpa* leaves, respectively. **LB, MB:** *n*-Butanol fractions of *T. laxiflora* and *T. myriocarpa* leaves, respectively.

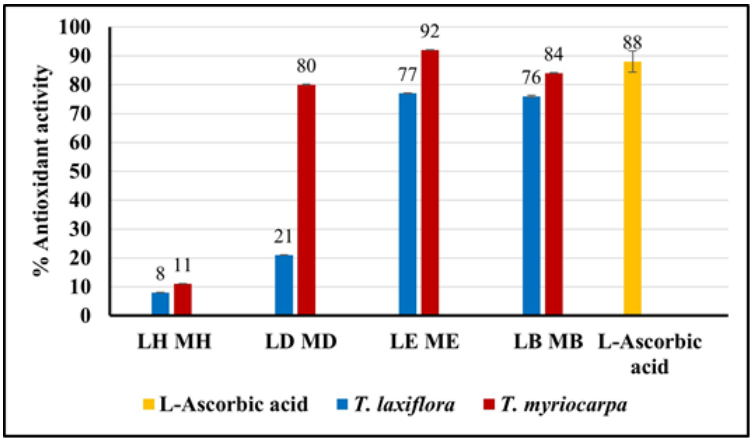

Fig. 1. Percentages of antioxidant activity (AA%) of *T. laxiflora* and *T. myriocarpa* leaf fractions estimated by DPPH radical scavenging assay

Table II: Inhibition zone diameters produced by T. laxiflora and T. myriocarpa leaf fractions using agar well diffusion method

LH, MH:

Fraction (100 mg/mL)	Microorganism inhibition zone diameter (mm)				
	Gram +ve bacteria		Gram –ve bacteria		Fungi
	Bacillus	*Staphylococcus*	*Escherichia*	*Klebsiella*	*Candida*
LH	10 ± 0.5	**17** ± 0.85	0	**14** ± 0.7	**13** ±
MH	0	16 ± 0.8	0	**13** ± 0.65	12 ± 0.6
LD	12 ± 0.6	13 ± 0.65	0	**12** ± 0.6	**14** ± 0.7
MD	0	10 ± 0.5	0	0	0
LE	12 ± 0.6	**11** ± 0.55	12 ± 0.6	**12** ± 0.6	**14** ± 0.7
ME	13 ±	16 ± 0.8	12 ± 0.6	**13** ± 0.65	13 ±
LB	**11** ±	0	12 ± 0.6	**13** ± 0.65	13 ±
MB	**15** ±	10 ± 0.5	0	**13** ± 0.65	16 ± 0.8
Ciprofloxacin	**28** ± 1.4	**30** ±1.5	25 ± 1.25	**33** ± 1.65	0
Fluconazole	0	0	0	0	**30** ± 1.5

Unsaponifiable matters of T. laxiflora and T. myriocarpa leaves, respectively. LD, MD: Dichloromethane fractions of T. laxiflora and T. myriocarpa leaves, respectively. LE, ME: Ethyl acetate fractions of T. laxiflora and T. myriocarpa leaves, respectively. LB, MB: n-Butanol fractions of T. laxiflora and T. myriocarpa leaves, respectively.

1.2. Antimicrobial activity

All the tested *T. laxiflora* and *T. myriocarpa* leaf fractions exhibited antimicrobial properties, as opposed to the previously reported lack of antibacterial activity of *T. laxiflora* crude methanolic leaf extract [11]. This might be explained by the fact that the fractions contained higher amounts of metabolites with antibacterial potential than the crude extract. The LE and ME fractions showed antimicrobial potential against all the examined gram-positive bacterial, gram-negative bacterial, and fungal strains **(Table II and Figure 2)**. Furthermore, the MIC values acquired using the broth macrodilution method correlated well with the observed diameters of inhibition zones, where the fractions exhibiting the largest diameters of inhibition zones also had the lowest MIC values **(Table III and Figure 3)**. The *n*-butanol fraction of *T. myriocarpa* leaf extract (MB) exhibited the strongest inhibition against *Bacillus subtilis* and *Candida albicans* with MICs equal to 6.7 ± 0.34 and 6.7 ± 0.33 mg/mL, respectively. Meanwhile, the most potent inhibition of *Staphylococcus aureus* was exhibited by the unsaponifiable matter of *T. laxiflora* leaves (LH) and the ME fraction with MICs of 6.42 ± 0.32 and 6.35 ± 0.32 mg/mL, respectively. Also, the most potent inhibition of *Klebsiella pneumonia* was exhibited by the LH and ME fractions with MICs of 12.83 ± 0.64 and 12.7 ± 0.64 mg/mL, respectively. The results also showed that the LE fraction exhibited the strongest anti-*Escherichia coli* activity with MIC equal to 13.15 ± 0.65 mg/mL.

Table III: MIC of *T. laxiflora* and *T. myriocarpa* leaf fractions against the test microorganisms

Sample	MIC (mg/mL)				
	Gram +ve bacteria		Gram –ve bacteria		Fungi
	Bacillus subtilis	*Staphylococcus*	*Escherichia coli*	*Klebsiella*	*Candida albicans*
LH	25.6 ± 1.28	6.42 ± 0.32	51.35 ± 2.57	12.83 ± 0.64	12.83 ± 0.64
MH	56.85 ± 2.84	7.11 ± 0.35	56.85 ± 2.84	14.22 ± 0.71	28.42 ± 1.42
LD	25.15 ± 1.26	12.6 ± 0.63	50.3 ± 2.5	25.15 ± 1.26	12.57 ± 0.63
MD	52.6 ± 2.63	26.3 ± 1.32	52.6 ± 2.63	25.4 ± 1.27	52.6 ± 2.63
LE	27.75 ± 1.39	27.75 ± 1.39	13.15 ± 0.65	13.15 ± 0.68	13.15 ± 0.66
ME	12.7 ± 0.63	6.35 ± 0.32	25.4 ± 1.27	12.7 ± 0.64	12.7 ± 0.63
LB	27.9 ± 1.39	55.9 ± 2.8	27.9 ± 1.39	27.9 ± 1.4	27.9 ± 1.39
MB	6.7 ± 0.34	27 ± 1.35	54.05 ± 2.70	13.5 ± 0.68	6.7 ± 0.33
Ciprofloxacin	1.12 ± 0.06	0.78 ± 0.04	1.56 ± 0.08	0.13 ± 0.007	-ve
Fluconazole	-ve	-ve	-ve	-ve	1.56 ± 0.08

LH, MH: Unsaponifiable matters of *T. laxiflora* and *T. myriocarpa* leaves, respectively. **LD, MD:** Dichloromethane fractions of *T. laxiflora* and *T. myriocarpa* leaves, respectively. **LE, ME:** Ethyl acetate fractions of *T. laxiflora* and *T. myriocarpa* leaves, respectively. **LB, MB:** *n*-Butanol fractions of *T. laxiflora* and *T. myriocarpa* leaves, respectively.

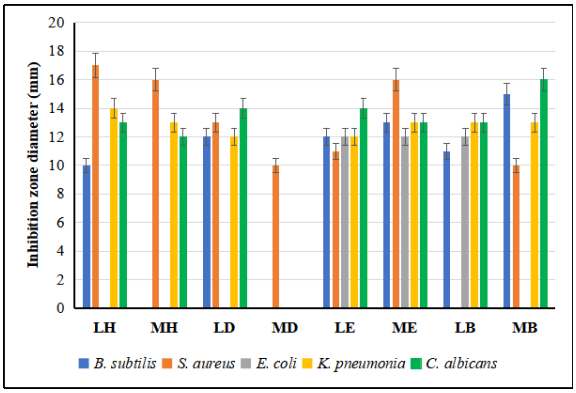

Fig. 2. Inhibition zone diameters of the unsaponifiable matters, dichloromethane, ethyl acetate, and *n*-butanol fractions of *T. laxiflora* and *T. myriocarpa* leaves against five microbial strains

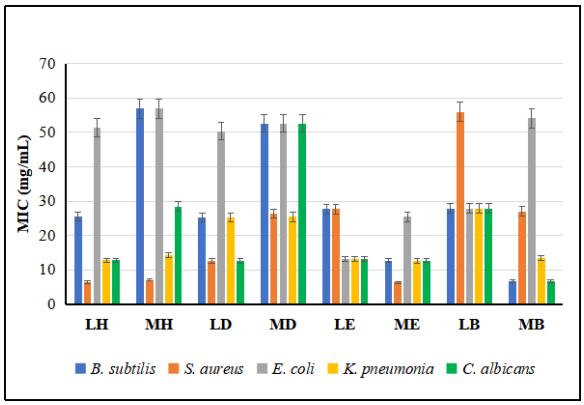

Fig. 3. MIC values of the unsaponifiable matters, dichloromethane, ethyl acetate, and *n*-butanol fractions of *T. laxiflora* and *T. myriocarpa* leaves against five microbial strains

1.3. Characterization of the chemical constituents

Identification of the components of each fraction that could account for its observed antioxidant and/or antimicrobial effects was carried out. The constituents of the unsaponifiable matters of *T. laxiflora* and *T. myriocarpa* leaves (LH and MH, respectively) were analyzed by GC-MS leading to the tentative identification of twenty-five and twenty-nine constituents, respectively, after comparing their fragmentation patterns with those reported in the Wiley library **(Table IV, Figures 4 and 5)**. As for the more polar fractions, namely the dichloromethane, ethyl acetate, and *n*-butanol fractions of *T. laxiflora* leaf extract (LD, LE, and LB, respectively), as well as the dichloromethane, ethyl acetate, and *n*-butanol fractions of *T. myriocarpa* leaf extract (MD, ME, and MB, respectively), the identification of their constituents was performed by comparing their ESI-MS spectra, obtained using the negative ion acquisition mode, with those reported for the compounds previously identified from *Terminalia* species. This resulted in the tentative identification of twenty-three and twenty-six constituents in *T. laxiflora* and *T. myriocarpa* leaf fractions, respectively **(Tables V and VI, Figures 6 and 7)**. However, isomeric structures with the same masses but different substituent locations or stereochemistry could not be unambiguously distinguished. Moreover, the identity of several compounds was established after their chromatographic isolation and spectral characterization *via* extensive 1D and 2D NMR analyses, as indicated by the authors in previous studies [12,13].

1.3.1. Characterization of the constituents of the LH and MH fractions

The LH and MH fractions contained significant amounts of hydrocarbons reaching up to 32.07 and 53.57 %, respectively. These were predominated by squalene in the LH fraction, representing 23.13 % of its total composition, and by tetratriacontane in the MH fraction, representing 9.43 % of its total composition. Also, several aliphatic alcohols and ketones were identified in the LH and MH fractions, collectively representing 23.83 and 15.82 %, respectively, of their total composition. Phytol and 9-hexadecen-1-ol predominated this group in the LH and MH fractions, respectively, with 5.26 and 4.82 % of their overall composition. The LH and MH fractions also contained phenols that represented 0.92 and 1.03 %, respectively, of their total content. Moreover, sterols were identified in the LH and MH fractions, constituting 11.43 and 6.74

%, respectively, of their total composition, with *β*-sitosterol being the most prevalent sterol in both fractions (8.05 and 4.57 %, respectively, of their overall composition). Additionally, triterpenoids were identified in the LH fraction constituting 15.32 % of its composition, with lupeol being the predominant terpenoid accounting for 13.03 % of the total LH content. In contrast, no triterpenoids were detected in the MH fraction.

Table IV: Results of GC/MS analysis of the LH and MH fractions

No.	R_t (min.)	Identified components	M^+	Molecular formula	% in LH	% in MH
1	6.77	2,5-Dimethylnonane	156	$C_{11}H_{24}$	n.d.	1.66
2	6.96	2-Methylundecane	170	$C_{12}H_{26}$	n.d.	1.21
3	7.41	4,8-Dimethylundecane	184	$C_{13}H_{28}$	n.d.	2.73
4	7.50	2,6,7-Trimethyldecane	184	$C_{13}H_{28}$	n.d.	1.54
5	7.57	Dodecane	170	$C_{12}H_{26}$	n.d.	2.19
6	7.76	2,6,10-Trimethyldodecane (Farnesane)	212	$C_{15}H_{32}$	n.d.	2.65
7	7.99	4,5-Dimethylnonane	156	$C_{11}H_{24}$	n.d.	1.04
8	8.17	4,6-Dimethylundecane	184	$C_{13}H_{28}$	n.d.	1.26
9	8.21	4-Ethyloctane	142	$C_{10}H_{22}$	n.d.	1.39
10	8.57	2,4-Dimethyldecane	170	$C_{12}H_{26}$	n.d.	1.54
11	8.63	1,2,4,5-Tetramethylbenzene (Durene)	134	$C_{10}H_{14}$	1.05	n.d.
12	9.59	1,2,3,4-Tetramethylbenzene	134	$C_{10}H_{14}$	0.71	n.d.
13	10.72	1-Dodecene	168	$C_{12}H_{24}$	n.d.	1.06
14	17.25	1-Tetradecene	196	$C_{14}H_{28}$	n.d.	3.59
15	20.00	1-Dodecanol	186	$C_{12}H_{26}O$	4.14	n.d.
16	20.94	2-Tertbutyl-4-isopropyl-5-methylphenol	206	$C_{14}H_{22}O$	n.d.	1.03
17	23.02	1-Tridecanol	200	$C_{13}H_{28}O$	3.74	n.d.
18	23.37	9-Hexadecen-1-ol	240	$C_{16}H_{32}O$	n.d.	4.82
19	28.93	1-Octadecanol	270	$C_{18}H_{38}O$	n.d.	4.38
20	28.96	Eicosane	282	$C_{20}H_{42}$	0.86	n.d.
21	30.14	6,10,14-Trimethyl-2-Pentadecanone	268	$C_{18}H_{36}O$	1.66	n.d.
22	33.95	1-Docosene	308	$C_{22}H_{44}$	n.d.	4.00
23	36.62	3,7,11,15-Tetramethyl--2-hexadecen-1-ol (Phytol)	296	$C_{20}H_{40}O$	5.26	3.65
24	38.54	1-Nonadecene	266	$C_{19}H_{38}$	n.d.	2.33
25	40.73	Heneicosane	296	$C_{21}H_{44}$	0.94	n.d.
26	42.75	1-Tetracosanol	354	$C_{24}H_{50}O$	n.d.	1.96
27	44.79	Pentacosane	352	$C_{25}H_{52}$	0.82	n.d.
28	46.67	Octacosanol	410	$C_{28}H_{58}O$	n.d.	1.01
29	46.72	Hexacosane	366	$C_{26}H_{54}$	0.77	n.d.
30	48.58	Nonacosane	408	$C_{29}H_{60}$	1.40	n.d.
31	48.74	Tetratetracontane	618	$C_{44}H_{90}$	n.d.	5.05
32	50.47	Heptacosane	380	$C_{27}H_{56}$	n.d.	2.30
33	50.59	Squalene	410	$C_{30}H_{50}$	23.13	2.57
34	52.13	Hentriacontane	436	$C_{31}H_{64}$	2.39	n.d.
35	52.43	2,6,10,15,19,23-Hexamethyl-1,6,10,14,18,22-tetracosahexaen-3-ol	426	$C_{30}H_{50}O$	3.18	n.d.
36	52.50	1-Hexacosene	364	$C_{26}H_{52}$	n.d.	4.20
37	52.61	2,2-Dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-oxirane	426	$C_{30}H_{50}O$	1.23	n.d.
38	52.82	3,7,11,15-Tetramethyl-2,6,10,14-hexadecatetraen-1-ol	290	$C_{20}H_{34}O$	1.33	n.d.
39	53.56	Geranylgeraniol	290	$C_{20}H_{34}O$	1.30	n.d.
40	53.74	1-Heptatriacontanol	536	$C_{37}H_{76}O$	2.09	n.d.
41	53.83	Pentatriacontane	492	$C_{35}H_{72}$	n.d.	1.83
42	55.64	1-Heptacosanol	396	$C_{27}H_{56}O$	1.13	n.d.
43	55.73	Tetratriacontane	478	$C_{34}H_{70}$	n.d.	9.43
44	55.89	*α*-Tocopherol	430	$C_{29}H_{50}O_2$	0.92	n.d.
45	57.77	Stigmasta-5,22-dien-3-ol (Stigmasterol)	412	$C_{29}H_{48}O$	n.d.	1.18
46	58.80	Stigmast-5-en-3-ol (*β*-Sitosterol)	414	$C_{29}H_{50}O$	8.05	4.57
47	59.26	*α*-Amyrin	426	$C_{30}H_{50}O$	1.43	n.d.
48	60.16	Lupeol	426	$C_{30}H_{50}O$	13.03	n.d.
49	60.74	24-Methylene-9,19-cyclolanost-24-en-3-ol	440	$C_{31}H_{52}O$	3.38	0.99
50	63.97	Olean-12-en-3-one	424	$C_{30}H_{48}O$	0.86	n.d.
Total % of identified components					84.8	77.16
Total number of identified components					25	29

R_t: Retention time, **M^+**: Molecular ion peak, **LH**, **MH**: Unsaponifiable matters of *T. laxiflora* and *T. myriocarpa* leaves, respectively. **n.d.**: Not detected.

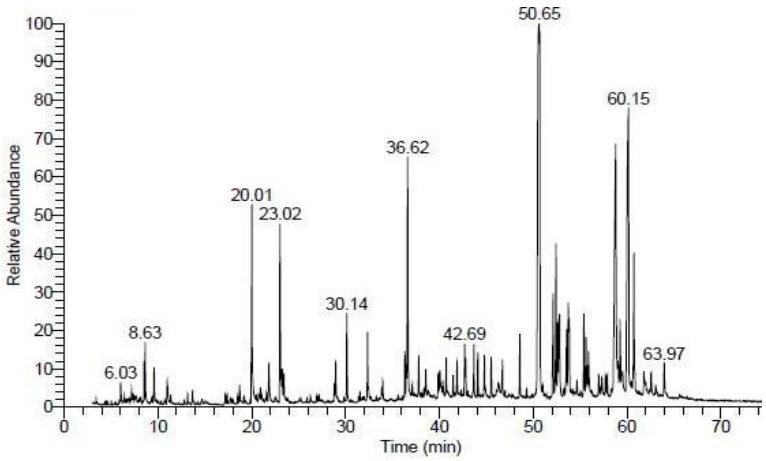

Fig. 4. Total ion current GC–MS chromatogram of the LH fraction

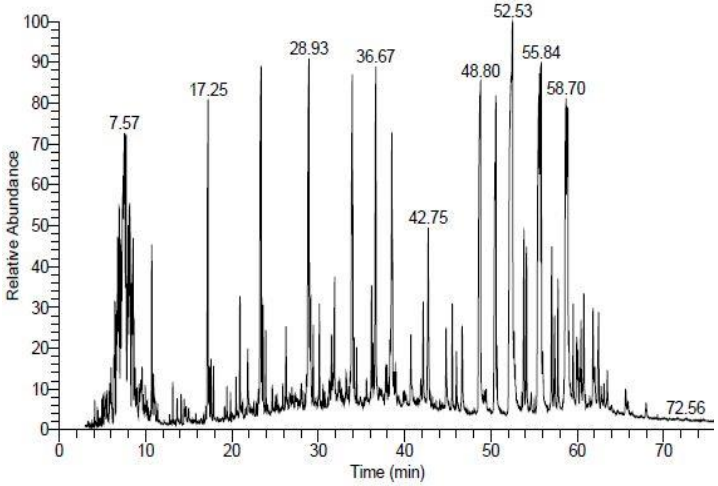

Fig. 5. Total ion current GC–MS chromatogram of the MH fraction

1.3.2. Characterization of the constituents of the LD, LE, LB, MD, ME, and MB fractions

1.3.2.1. Characterization of tannins

As for *T. laxiflora* leaf fractions, tannins were detected only in the LE and LB, accounting for 8.13 and 20.58 % of their composition, respectively. A total of nine tannins were detected. These included simple acids, gallate esters, gallotannins, and ellagitannins. Among these, corilagin and chebulagic acid were isolated from the LB fraction, and their structures were elucidated by comparing their ^{1}H- and ^{13}C-NMR spectral data to those in the literature [12]. On the other hand, three methyl esters of tannins were detected in the MD fraction, constituting 8.52 % of its composition. In addition, a total of ten tannins were detected in the ME and MB fractions, accounting for 20.51 and 20.05 %, respectively, of their composition. Most gallotannins provided $[M-H-169]^-$ and $[M-H-152]^-$ fragment ions, suggesting the loss of gallic acid and galloyl moieties, respectively. Moreover, other fragment ions, including $[M-H-169-169]^-$, $[M-H-152-152]^-$, and $[M-H-152-301]^-$ were observed due to the sequential loss of gallic acid, galloyl, and hexahydroxydiphenoyl (HHDP) moieties. Also, the observation of fragment ions at m/z 169 and 301 confirmed the presence of gallic acid and HHDP fragments, respectively [14]. In addition, the molecular ion $[M-2H]^{2-}$ characteristic for ESI mass spectra was also observed [15]. Furthermore, the identified ellagitannin, *viz.*,ellagic acid hexoside, produced $[M-H-162]^-$ fragment ion, indicating the loss of a hexose sugar moiety [14].

1.3.2.2. Characterization of flavonoid glycosides

Concerning *T. laxiflora* leaf fractions, flavonoid glycosides were exclusively detected in the LB fraction and represented 3.19 % of its composition. The two flavonoid glycosides rutin and orientin were identified. The base peaks of their mass spectra were found to be the $[M-H]^-$ ions. Additionally, the cleavage of the aglycone moiety of rutin (flavonoid-*O*-glycoside) was evident from the fragment ion observed at m/z 301 [16], while the aglycone cleavage did not occur in the case of orientin as expected for a flavonoid-*C*-glycoside [17]. Meanwhile, a total of three flavonoid-*C*-glycosides were identified in the ME and

MB fractions, representing 4.5 and 13.21 %, respectively, of their composition. Among the detected flavonoid glycosides, rutin and isovitexin were formerly isolated from *T. laxiflora* and *T. myriocarpa* leaf extracts, respectively, and identified by the comparison of their ^{1}H- and ^{13}C-NMR spectral data to literature values [11,13].

1.3.2.3. Characterization of triterpene glycosides

Among the fractions of *T. laxiflora* leaves, triterpene glycosides were found only in the LB fraction. This group included three compounds and made up 1.16% of the fraction. From this group, arjunglucoside II and chebuloside II were isolated by the authors from the hydromethanolic leaf extract, and their structures were elucidated after the comparison of their ^{1}H- and ^{13}C-NMR spectral data to literature values [12]. In contrast, triterpene glycosides were discovered to be more prevalent in *T. myriocarpa* leaves, where they constituted 10.33, 33.84, and 12.75 %, respectively, of MD, ME, and MB composition. Some of the identified compounds, *viz.* nigaichigoside F1, quadranoside IV, and rosamultin, were also isolated from the ME fraction and identified by comparing their ^{1}H- and ^{13}C-NMR spectral data to literature reports [13]. Interestingly, all the triterpene glycosides gave the adduct $[M+formic\ acid-H]^-$ as their base peaks, in accordance with literature reports [15,16].

2.3.2.4. Characterization of lignan glycosides

Two dibenzylbutane lignan glucosides, namely laxilignans B and C, were recognized in the LE and LB fractions, accounting for 1.03 and 0.55 %, respectively, of their composition. According to a prior investigation by the authors, the identity of these lignan glucosides was determined following their chromatographic separation and spectral characterization using comprehensive 1D and 2D NMR analyses [12]. Pseudo-molecular ion peaks $[M-H]^-$ matching the masses of laxilignans B and C were observed. Furthermore, the molecular ion adducts $[2M-H]^-$ and $[M+formic\ acid-H]^-$, which are frequently detected in the negative ionization ESI mass spectra [15], were also found. However, no lignan glycosides were detected in *T. myriocarpa* leaf fractions.

2.3.2.5. Characterization of lignans

The LD fraction was found to contain three lignans representing 2.48 % of its composition. One tetrahydrofuran lignan and two dibenzylbutane lignans were identified based on the pseudo-molecular ion peaks [15]. These were also isolated from the fraction and identified based on their NMR spectral data [12]. On the other hand, only termilignan B was detected in the MD fraction, accounting for 2.08 % of its composition, whereas the ME and MB were found to contain no lignans.

2.3.2.6. Characterization of triterpene acids

Four peaks that were observed in the UPLC chromatograms of the LD, LE, and LB fractions were assignable for triterpene acids constituting 8.33, 3.99, and 5.31 %, respectively, of their composition. Of these, asiatic and arjunolic acids were previously isolated as a mixture from the LE fraction and identified based on their ^{1}H- and ^{13}C-NMR spectral data [12]. Similarly, six peaks in the UPLC chromatograms of the MD, ME, and MB fractions were attributed to triterpene acids, where they constituted 15.92, 9.11 , and 8.24 %, respectively, of their composition. Among these, 19α-hydroxyasiatic, asiatic, and arjunic acids were isolated from the ME fraction, and alphitolic acid was isolated from the MD fraction, followed by structural elucidation by comparing their ^{1}H- and ^{13}C-NMR spectral data to literature values [13]. The $[M+formic\ acid-H]^-$ adducts were observed in the mass spectra of all the identified triterpene acids [15], albeit in much lower abundances than those of the $[M-H]^-$ ions.

Table V: Constituents identified by UPLC–ESI–MS analysis in the LD, LE, and LB fractions using the negative ion acquisition mode

Peak no.	R_t (min.)	Area%			[M-H]$^-$ (m/z)	[2M-H]$^-$/ [M-2H]$^{2-}$/ [M+FA-H]$^-$ (m/z)	Identified compound	Molecular formula	Exact mass (g/mol)	Fragment ions (m/z)	References
		LD	LE	LB							
I. Tannins											
1	0.78	n.d.	1.87	6.12	785.2	n.d./ 392.5/ n.d.	Digalloyl HHDP glucose isomer I[a]	$C_{34}H_{26}O_{22}$	786.092	633 [M-H-G]$^-$, 635, 636, 481 [M-H-2G]$^-$, 331 [M-H-G-HHDP]$^-$, 169 [GA-H]$^-$	[14]
2	0.99	n.d.	n.d.	0.81	331.2	n.d./ n.d./ n.d.	Monogalloyl glucose isomer I[a]	$C_{13}H_{16}O_{10}$	332.074	313 [M-H-H_2O]$^-$, 297, 267, 203, 173, 169 [M-H-Glc]$^-$,	[18]

Table V: continued

										133, 113	
3	1.04	n.d.	1.94	0.93	169.1	n.d./ n.d./ n.d.	Gallic acid[b]	$C_7H_6O_5$	170.022	129	[11]
4	1.06	n.d.	n.d.	0.93	331.1	n.d./ n.d./ n.d.	Monogalloyl glucose isomer II[a]	$C_{13}H_{16}O_{10}$	332.074	313 [M-H-H_2O], 297, 267, 173, 169 [M-H-Glc]$^-$, 133, 113	[18]
5	3.06	n.d.	0.93	n.d.	183.1	n.d./ n.d./ n.d.	Methyl gallate[b]	$C_8H_8O_5$	184.037	179	[11]
6	5.33	n.d.	1.65	9.74	633.2	n.d./ n.d./ n.d.	Corilagin[b]	$C_{27}H_{22}O_{18}$	634.081	432, 431	[12, 14]
7	6.13	n.d.	0.53	1.33	953.2	n.d./ 476.2/ n.d.	Chebulagic acid[b]	$C_{41}H_{30}O_{27}$	954.097	787, 786, 785 [M-H-GA]$^-$, 616, 615 [M-H-2GA]$^-$, 487, 392, 329	[12, 14]
8	6.27	n.d.	0.48	0.35	785.2	n.d./ 392.2/ n.d.	Digalloyl HHDP glucose isomer II[a]	$C_{34}H_{26}O_{22}$	786.092	713, 617, 616, 615 [M-H-GA]$^-$, 609, 492, 301 [M-H-G-GA-Glc]$^-$	[14]
9	6.51	n.d.	0.73	0.37	463.2	n.d./ n.d./ n.d.	Ellagic acid hexoside[a]	$C_{20}H_{16}O_{13}$	464.059	449, 301 [M-H-hexoside], 241, 173	[9]
II. Flavonoid glycosides											
10	5.99	n.d.	n.d.	1.01	447.3	n.d./ n.d./ n.d.	Orientin[a]	$C_{21}H_{20}O_{11}$	448.101	371, 305	[16]
11	6.37	n.d.	n.d.	2.18	609.3	n.d./ n.d./ n.d.	Rutin[b]	$C_{27}H_{30}O_{16}$	610.153	475, 431, 301 [M-H-Glc-Rha]$^-$	[11]
III. Triterpene glycosides											
12	7.71	n.d.	n.d.	0.36	n.d.	n.d./ n.d./ 711.6	Arjunglucoside I[a]	$C_{36}H_{58}O_{11}$	666.398	512, 503 [M-H-Glc]$^-$, 476, 303	[19, 20]
13	9.01	n.d.	n.d.	0.33	n.d.	n.d./ n.d./ 711.6	Chebuloside II[b]	$C_{36}H_{58}O_{11}$	666.398	579, 551, 431, 388, 179	[12, 21]
14	9.48	n.d.	n.d.	0.47	n.d.	n.d./ n.d./ 695.7	Arjunglucoside II[b]	$C_{36}H_{58}O_{10}$	650.403	575, 535, 431, 327	[12, 20]
IV. Lignan glycosides											
15	8.09	n.d.	0.68	0.55	489.2	979.5/ n.d./ 535.3	Laxilignan C[b]	$C_{25}H_{30}O_{10}$	490.184	445	[12]
16	9.08	n.d.	0.35	n.d.	473.3	947.6/ n.d./ 519.3	Laxilignan B[b]	$C_{25}H_{30}O_9$	474.189	n.d.	[12]

Table V: continued

V. Lignans											
17	10.92	0.79	n.d.	n.d.	311.2	623.4/ n.d./ 357.2	Laxilignan A[b]	$C_{19}H_{20}O_4$	312.136	299	[12]
18	13.97	0.65	n.d.	n.d.	299.3	n.d./ n.d./ 345.3	Termitomenin C[b]	$C_{18}H_{20}O_4$	300.136	181, 179, 177	[12]
19	14.95	1.04	n.d.	n.d.	293.3	n.d./ n.d./ n.d.	Termilignan B[b]	$C_{19}H_{18}O_3$	294.126	265, 135	[12]
VI. Triterpene acids											
20	12.53	0.34	1.43	1.10	487.5	975.8/ n.d./ 533.5	Asiatic acid[b] and/or ajunolic acid[b]	$C_{30}H_{48}O_5$	488.350	311	[12, 22]
21	13.33	1.53	n.d.	n.d.	487.5	976.1/ n.d./ 533.5	Triterpene acid isomer I[a]	$C_{30}H_{48}O_5$	488.350	310, 309, 194	[23]
22	15.94	4.18	1.25	3.83	471.5	943.9/ n.d./ 517.5	Triterpene acid isomer II[a]	$C_{30}H_{48}O_4$	472.355	431, 310, 309, 295, 289, 174	[22, 24]
23	16.14	2.28	1.31	0.38	471.5	944.3/ n.d./ 517.5	Triterpene acid isomer III[a]	$C_{30}H_{48}O_4$	472.355	430, 312, 311, 293	[22, 24]

[a]Previously reported in the genus *Terminalia*, [b]Isolated from *T. laxiflora* hydromethanolic leaf extract, **LD, LE, LB:** Dichloromethane, ethyl acetate, and *n*-butanol fractions of *T. laxiflora* hydromethanolic leaf extract, respectively. **FA:** Formic acid, **G:** Galloyl moiety, **HHDP:** Hexahydroxydiphenoyl moiety, **Glc:** Glucosyl moiety, **GA:** Gallic acid moiety, **Rha:** Rhamnosyl moiety, **n.d.:** Not detected.

Table VI: Constituents identified by UPLC–ESI–MS analysis in the MD, ME, and MB fractions using the negative ion acquisition mode

Peak no.	R_t (min.)	Area%			[M-H]$^-$ (m/z)	[2M-H]$^-$ / [M-2H]$^{2-}$/ [M+FA-H]$^-$ (m/z)	Identified compound	Molecular formula	Exact mass (g/mol)	Fragment ions (m/z)	References
		MD	ME	MB							
I. Tannins											
1	0.78	n.d.	3.42	n.d.	785.2	n.d./ 392.7/ n.d.	Digalloyl HHDP glucose isomer[a]	$C_{34}H_{26}O_{22}$	786.092	636, 635, 633 [M-H-G]$^-$, 483, 482, 481 [M-H-2G]$^-$, 169 [GA-H]$^-$	[14]
2	0.80	n.d.	n.d.	4.95	481.2	963.2/ n.d./ n.d.	HHDP glucose isomer I[b]	$C_{20}H_{18}O_{14}$	482.07	377, 331 [GG-H]$^-$, 219, 217, 169 [GA-H]$^-$, 163	[25]
3	0.99	n.d.	n.d.	0.81	481.2	963.4/ 239.3/ n.d.	HHDP glucose isomer II[b]	$C_{20}H_{18}O_{14}$	482.07	332, 331 [M-H-G]$^-$, 173, 169 [GA-H]$^-$	[25]
4	1.02	n.d.	5.52	n.d.	169.1	339.1/ n.d./ n.d.	Gallic acid[b]	$C_7H_6O_5$	170.022	n.d.	[25]
5	1.39	n.d.	n.d.	4.76	783.1	n.d./ 391.3/ n.d.	Di-HHDP glucose isomer I[a]	$C_{34}H_{24}O_{22}$	784.076	613 [M-H-GA]$^-$, 591, 542, 541, 470, 469 [M-H-G-	[14]

Table VI: continued

										Glc]$^-$, 279	
6	1.93	n.d.	n.d.	2.05	783.3	n.d./ n.d./ n.d.	Di-HHDP glucose isomer II[a]	$C_{34}H_{24}O_{22}$	784.076	634, 633, 541	[14]
7	2.76	0.45	7.89	n.d.	183.2	367.2/ n.d./ n.d.	Methyl gallate[b]	$C_8H_8O_5$	184.037	n.d.	[25]
8	5.54	n.d.	n.d.	1.46	483.2	n.d./ n.d./ n.d.	Digalloyl glucose isomer I[a]	$C_{20}H_{20}O_{14}$	484.085	431, 421, 323, 247, 179 [M-H-2G]$^-$, 113	[26]
9	5.68	n.d.	2.67	2.57	483.2	967.2/ n.d./ n.d.	Digalloyl glucose isomer II[a]	$C_{20}H_{20}O_{14}$	484.085	306, 305, 293, 225	[26]
10	9.95	4.40	n.d.	n.d.	329.4	n.d./ n.d./ n.d.	Dimethyl ellagic acid[a]	$C_{16}H_{10}O_8$	330.038	301 [M-H-2Me]$^-$	[27]
11	17.06	3.67	1.01	3.45	483.4	n.d./ n.d./ n.d.	Methyl flavogallonate[b]	$C_{22}H_{12}O_{13}$	484.027	433	[25]
II. Flavonoid glycosides											
12	5.97	n.d.	n.d.	2.05	447.2	895.4/ n.d./ n.d.	Orientin[a]	$C_{21}H_{20}O_{11}$	448.101	441, 305	[16]
13	6.08	n.d.	n.d.	0.79	447.2	895.4/ n.d./ n.d.	Isoorientin[a]	$C_{21}H_{20}O_{11}$	448.101	441, 403, 305	[16]
14	6.30	n.d.	4.50	10.37	431.3	863.4/ n.d./ n.d.	Isovitexin[b]	$C_{21}H_{20}O_{10}$	432.106	301	[13]
III. Triterpene glycosides											
15	7.64	n.d.	4.10	2.09	n.d.	n.d./ n.d./ 711.6	Nigaichigoside F1[b]	$C_{36}H_{58}O_{11}$	666.398	583, 525, 461, 351	[13]
16	9.15	2.19	11.19	2.89	n.d.	n.d./ n.d./ 695.6	Quadranoside IV[b]	$C_{36}H_{58}O_{10}$	650.403	599, 215	[13]
17	9.36	7.74	17.64	7.00	n.d.	n.d./ n.d./ 695.6	Rosamultin[b]	$C_{36}H_{58}O_{10}$	650.403	596, 361	[13]
18	11.60	0.40	0.91	0.77	n.d.	n.d./ n.d./ 677.6	Triterpene acid hexoside[a]	$C_{36}H_{56}O_9$	632.392	554, 553, 549, 533, 531, 503, 469 [M-H-Glc]$^-$, 179	[28]
IV. Flavonoids											
19	10.21	1.83	n.d.	n.d.	287.4	n.d./ n.d./ n.d.	Eriodictyol[a]	$C_{15}H_{12}O_6$	288.063	229, 179	[29]
V. Triterpene acids											
20	10.29	0.22	0.8	1.07	503.5	n.d./ n.d./ 549.5	19α-Hydroxy asiatic acid[b]	$C_{30}H_{48}O_6$	504.345	461, 355, 288, 287, 229, 209, 179	[13]
21	12.50	0.60	1.43	1.33	487.5	975.9/ n.d./ 533.5	Asiatic acid[b]	$C_{30}H_{48}O_5$	488.350	315, 309	[13]
22	12.92	6.17	5.13	2.53	487.5	975.9/ n.d./ 533.5	Arjunic acid[b]	$C_{30}H_{48}O_5$	488.350	n.d.	[13]

Table VI: continued

23	15.38	4.95	0.86	2.73	471.5	943.9/ n.d./ 517.5	Alphitolic acid[b]	$C_{30}H_{48}O_4$	472.355	291	[13]
24	15.78	2.65	0.89	0.58	471.5	943.9/ n.d./ 517.5	Maslinic acid[a]	$C_{30}H_{48}O_4$	472.355	311, 309	[24]
25	18.91	1.33	n.d.	n.d.	455.5	912.4/ n.d./ 501.5	Triterpene acid[a]	$C_{30}H_{48}O_3$	456.360	435, 339	[29]
VI. Lignans											
26	14.83	2.08	n.d.	n.d.	293.4	n.d./ n.d./ n.d.	Termilignan B[a]	$C_{19}H_{18}O_3$	294.126	265	[12]

[a]Previously reported in the genus *Terminalia*, [b]Isolated from *T. myriocarpa* hydromethanolic leaf extract, **MD, ME, MB:** Dichloromethane, ethyl acetate, and *n*-butanol fractions of *T. myriocarpa* hydromethanolic leaf extract, respectively, **FA:** Formic acid, **Glc:** Glucosyl moiety, **HHDP:** Hexahydroxydiphenoyl moiety, **GA:** Gallic acid moiety, **G:** Galloyl moiety, **GG:** Galloyl glucose moiety, **Me:** Methyl moiety, **n.d.**: Not detected.

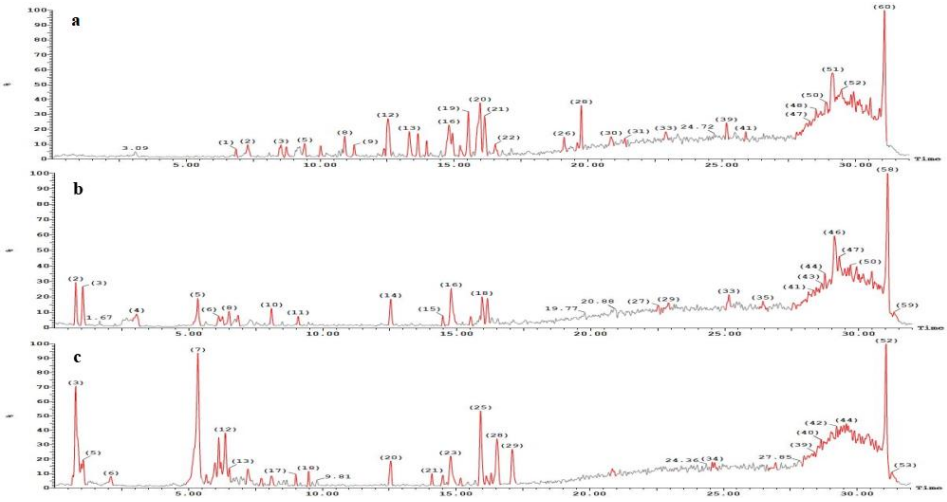

Fig. 6. Base peak UPLC–ESI–MS chromatograms of the (a) dichloromethane fraction, (b) ethyl acetate fraction, and (c) *n*-butanol fraction of the hydromethanolic extract of *T. laxiflora* leaves in the negative ion acquisition mode

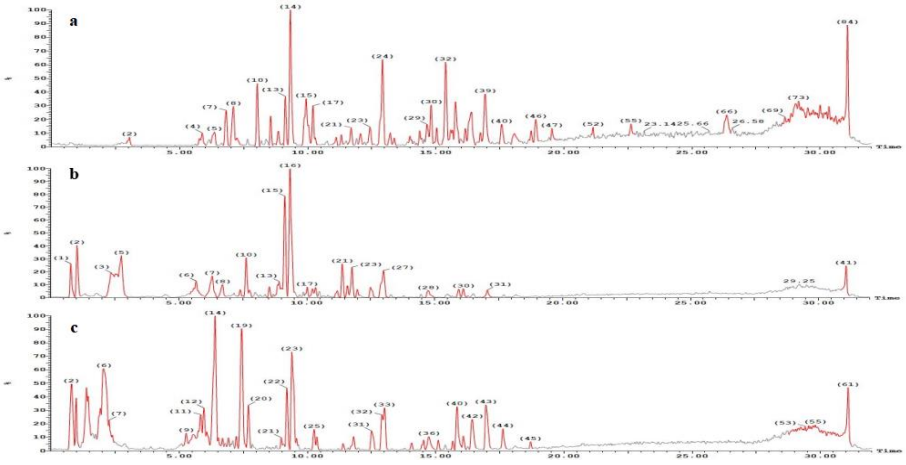

Fig. 7. Base peak UPLC–ESI–MS chromatograms of (a) dichloromethane fraction, (b) ethyl acetate fraction, and (c) *n*-butanol fraction of the hydromethanolic extract of *T. myriocarpa* leaves in the negative ion acquisition mode

2.4. Correlation between the antioxidant potential of the fractions and their chemical composition

The antioxidant capability of the LE and LB fractions could be explained by the reported antioxidant activity of their identified tannin and triterpene acid constituents, *viz.*, monogalloyl HHDP glucose isomer [30], chebulagic acid [30], asiatic acid [31], and arjunolic acid [32]. Also, the antioxidant activity of the ME fraction might be referred to the significant antioxidant potential of the constituents identified therein which included gallic acid, methyl gallate [30], methyl flavogallonate [33], asiatic acid [31], and maslinic acid [34]. The antioxidant capacity of tannins and triterpene acids could be ascribed to their hydrogen-donor hydroxyl groups [30]. The superior antioxidant potential of the ME fraction, compared to that of the LE and LB fractions, could be ascribed to its large content of gallic acid and methyl gallate which made up 13.41 % of the fraction's composition *versus* 2.87 and 0.93 % of the LE and LB fractions, respectively. Gallic acid and methyl gallate might have augmented the antioxidant activity of ME owing to their high density of hydroxyls over molecular volume, high molecular mobility, and good 3D accessibility of hydroxyl groups that enhance their molar DPPH scavenging capacities compared to other polyphenolic compounds [30].

2.5. Correlation between the antimicrobial potential of the fractions and their chemical composition

The inhibition of *Bacillus subtilis* by the LB fraction could be explained by the anti-*Bacillus subtilis* activity of rosamultin [35] and asiatic acid [36] detected therein. As revealed by a previous study, the antibacterial action of asiatic acid depends on the presence of a hydroxyl group at C-23 [37].

The inhibitory effect of the ME fraction against *Staphylococcus aureus* might be attributed to its phenolic constituents *viz.*, gallic acid [38], methyl gallate [39] as well as its triterpenoidal constituents, namely asiatic acid [36], and maslinic acid [40]. Plant phenolics are believed to exert different mechanisms of antibacterial action from those of conventional antibiotics. They have the ability to hinder the formation of biofilms, decrease host ligand adherence, neutralize bacterial toxins, decrease bacterial membrane fluidity, prevent the synthesis of bacterial cell walls and nucleic acids, and inhibit bacterial energy metabolism [41]. The antibacterial activity of phenolic compounds and their interactions with cell membrane structures are significantly influenced by the location of their hydroxyl groups. The presence of hydroxyl groups in delocalized electron systems is assumed to cause cytoplasmic membrane destabilization and collapse of the proton motive force which ultimately results in microbial death. Additionally, phenolic hydroxyl groups can form hydrogen bonds with microbial enzyme active sites, diminishing the catalytic activity of the enzymes [41].

The anti-*Staphylococcus aureus* effect of the LH fraction could be ascribed to α-amyrin [42], lupeol [43], squalene [44], β-sitosterol [45], α-tocopherol [46], 1-dodecanol [47], geranylgeraniol, and phytol [48] identified therefrom. Of these, squalene, found abundantly in the LH fraction (23.13% of its composition) was reported to inhibit staphyloxanthin production in *Staphylococcus aureus*. Staphyloxanthin is a carotenoid expressed by *Staphylococcus aureus* to protect the pathogen from reactive oxygen species (ROS) damage. The reduction of Staphyloxanthin synthesis brought about by squalene renders *Staphylococcus aureus* more vulnerable to the damage caused by ROS [49].

The inhibitory activity exhibited by the LE fraction against *Escherichia coli* could be attributed to the synergistic anti-*Escherichia coli* effects of gallic acid [50], corilagin [51], asiatic acid, and arjunolic acid [37], identified threrein. Contrary to the common belief that Gram-negative bacteria are more resistant to antibacterial drugs than their Gram-positive counterparts, the LE fraction notably showed lower MIC values against Gram-negative bacteria as compared to Gram-positive ones. Gram-negative bacteria have an outer membrane that combines a highly hydrophobic lipid bilayer with pore-forming proteins that have size-exclusion features, acting as selective barriers. This membrane is thought to be the reason behind the bacteria's resistance to antibiotics [52]. Due to their hydrophobic nature, arjunolic acid and asiatic acid most likely utilize the lipid-mediated route to pass through the outer membrane of *Escherichia coli* and exhibit their antibacterial effects [52]. Gallic acid was observed to significantly reduce the capacity of *Escherichia coli* to survive by causing damage to both its outer and inner membranes and by inhibiting the mRNA expression of the membrane permeability-related genes acrA, acrB, tolC, acrD, and acrF [50]. Corilagin possibly inhibited the *Escherichia coli* growth by interfering with the permeability of their membranes [51].

The anti-*Klebsiella pneumonia* activity of the the ME fraction could be attributed to its gallic acid content [53]; whereas that of the LH fraction might be ascribed to both squalene [44] and β-sitosterol [45], identified therein. The inhibition of *Klebsiella pneumonia* by β-sitosterol could be explained by the ability of β-sitosterol to disrupt the microbial cell membrane [45].

The inhibitory action exerted by the MB fraction against *Candida albicans* could be justified by its antifungal metabolic components *viz.*, nigaichigoside F1 [35], rosamultin [35], asiatic acid [54], and maslinic acid [55]. This agrees with previous studies that have revealed that triterpene acids elicit an antifungal effect through the alteration of the membrane lipid composition, change of the membrane permeability, inhibition of the drug efflux pump, accumulation of reactive oxygen species (ROS), and inhibition of the hyphal growth [54,56].

2. Experimental

2.1. Plant material

The leaves of *T. laxiflora* Engl. and *T. myriocarpa* Van Heurck & Müll. Arg. were collected from Al-Zohriya Botanical Garden, Zamalek, Cairo, Egypt, and the Zoo Garden, Giza, Egypt, respectively, in March 2020. The plant material was identified by Ms. Therese Labib, the consultant taxonomist at Al-Orman and Al-Qubba Botanical Gardens. Voucher specimens of *T. laxiflora* and *T. myriocarpa* leaves bearing the identification M166 and M165, respectively, were placed in the herbarium of the National Research Centre, Giza, Egypt.

2.2. *Preparation of the leaf extracts and fractions*

A portion of the air-dried powdered leaves of of *T. laxiflora* and *T. myriocarpa* (50 g, each) was, separately, extracted by maceration with *n*-hexane (3 × 0.5 L, each) yielding 2.46 and 0.97 g of lipoidal matter, respectively. Refluxing each of the obtained *n*-hexane extracts with 25 mL of 5 % (w/v) alcoholic potassium hydroxide for 5 hours led to saponification of their saponifiable constituents. After the saponified samples were vacuum-evaporated, the residues were suspended in 50 mL of distilled water and extracted multiple times using dichloromethane (50 mL × 4). The combined dichloromethane fractions were extensively washed with distilled water until they became alkalinity-free, dried over anhydrous sodium sulphate, and vacuum-evaporated to yield 0.85 and 0.29 g of *T. laxiflora* and *T. myriocarpa* unsaponifiable matter residues (LH and MH), respectively [57].

In the meantime, another portion of the air-dried powdered leaves of each of *T. laxiflora* (3 kg) and *T. myriocarpa* (2.2 kg) was, separately, exhaustively extracted at room temperature using 100 % methanol and then 70 % methanol in distilled water. Each combined extract was vacuum-dried giving 410 and 258 g of *T. laxiflora* and *T. myriocarpa* hydromethanolic leaf extracts, respectively. A portion of each extract (100 g) was then, separately, suspended in distilled water (1 L) and partitioned with dichloromethane (1 L x 5), ethyl acetate (1 L x 5), and water-saturated *n*-butanol (1 L x 5), in succession. This partitioning afforded the combined dichloromethane fraction (LD) (9 g), ethyl acetate fraction (LE) (25 g), and *n*-butanol fraction (LB) (34 g). On the other hand, the partitioning of *T. myriocarpa* hydromethanolic leaf extract yielded the combined dichloromethane fraction (MD) (6 g), ethyl acetate fraction (ME) (9 g), and *n*-butanol fraction (MB) (11 g).

2.3. Evaluation of the antioxidant activity

The percentage of the antioxidant activity (AA%) of each fraction was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. This technique has been widely utilized to predict antioxidant activity due to its quick turnaround time for analysis. The reduction of DPPH concentration due to its scavenging by the antioxidant compounds was monitored by the decrease of deep violet color intensity (λmax = 517 nm). Each reaction mixture was prepared by adding 0.5 mL of the test sample (760 µg/mL), 3 mL of absolute methanol, and 0.3 mL of DPPH in methanol (0.5 mM). After incubating the reaction mixtures for 100 minutes, their absorbances were measured at 517 nm. A blank was prepared by adding 3.3 mL of methanol to 0.5 mL of the test sample. The negative control was prepared by mixing 3.5 mL of methanol with 0.3 mL of DPPH solution, while the positive control contained 3.5 mL of L-ascorbic acid in methanol (109 µg/mL) and 0.3 mL of DPPH solution. Results were obtained as means of three replicates ± standard deviations [58].

The percentage of DPPH scavenging (AA %) was determined according to the following formula [59]:

$$AA\ \% = 100 - [(Absorbance_{sample} / Absorbance_{Negative\ Control}) \times 100]$$

2.4. Evaluation of the antimicrobial activity

The antimicrobial activity of the test fractions was evaluated *in-vitro* by the agar well diffusion method. Suspensions of *Bacillus subtilis* (NRRL B-543), *Staphylococcus aureus* (NRRL B-313), *Escherichia coli* (NRRL B-210), *Klebsiella pneumonia* (NCIMB 10341), and *Candida albicans* (NRRL Y-477) were prepared from fresh slants in 3 mL sterile saline solution. The suspensions were then adjusted to 0.5 McFarland (1–2 × 108 cfu/mL) and inoculated uniformly (5 drops) in nutrient agar plates for the bacterial strains and Sabouraud dextrose agar (SDA) medium plates for the fungal strain. A cork borer was used to create 7 mm-diameter holes in the agar. Each studied fraction (0.1 mL) was added to a hole at a concentration of 100 mg/mL in dimethylsulfoxide. As a negative control, a dimethylsulfoxide-filled hole was utilized. As a positive control, a standard antibiotic drug, namely ciprofloxacin (100 µg/mL), and a standard antifungal drug, namely fluconazole (100 units/mL), were utilized for the antibacterial and antifungal activities, respectively. The plates were pre-incubated for 1 hour at room temperature to allow diffusion of the applied solutions and reduce the impact of variation in the time of application of the different solutions. The plates were then incubated for 24 hours at 35 °C, followed by the measurement of the inhibition zone diameters. Results were obtained as the mean value of three independent experiments ± standard deviation [60].

3.5. Determination of the minimum inhibitory concentrations

The minimum inhibitory concentrations (MICs) of the test fractions and control drugs against the relevant bacterial and fungal strains were determined using the broth macrodilution method. At 35 °C, the bacterial strains were grown aerobically

overnight on nutrient agar, while the fungal strain, *viz.*, *Candida albicans*, was grown aerobically for 48 hours on SDA. Afterward, suspensions of the bacterial and fungal strains were prepared in 3 mL sterile saline solution and adjusted to 0.5 McFarland ($1–2 \times 10^8$ cfu/mL). Stock solutions of the test fractions in dimethylsulfoxide were prepared at a concentration of 100 mg/mL. Then, each stock solution was serially diluted in the corresponding culture medium (nutrient broth for the antibacterial assay and Sabouraud dextrose broth for the antifungal assay) in test tubes to achieve concentrations ranging from 5 to 100 mg/mL. Finally, the different strains were inoculated at final concentrations of 5×10^5 cfu/mL. After the tubes had been incubated aerobically for 24 hours at 35 °C, the turbidity was visually assessed to determine the MICs. The MIC was identified as the concentration at which there was no turbidity at all following the designated incubation period. Dimethylsulfoxide was used as a negative control, whereas ciprofloxacin and fluconazole were used as positive controls in bacteria and fungi experiments, respectively. Results were obtained as the mean value of three independent experiments ± standard deviation [61].

3.6. Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS analyses of the LH and MH fractions were carried out using helium as a carrier gas at a flow rate of 1.0 mL/minute and a split ratio of 1:10. The temperature used was 55 °C for 1 minute, rising at 5 °C/minute to 300 °C, then held for 15 minutes. The injector and detector were held at 280 °C. A portion of each fraction (150 mg) was dissolved in hexane (1 mL), filtered to remove any insoluble material, diluted in hexane (1:10, v/v), and injected (0.2 µL) into the GC apparatus. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 35-550. The Wiley mass spectral library collection was used to identify the compounds by comparing their spectral data.

3.7. Ultra-performance Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (UPLC-ESI-MS) analysis

An aliquot (1 mg) of each of the LD, LE, LB, MD, ME, and MB fractions was, separately, dissolved in 1 mL of 100 % methanol. The resulting solutions underwent a 2-minute sonication, a 3-minute centrifugation at 13,000 rpm to exclude insoluble material, and an injection of the supernatants into the UPLC apparatus. The UPLC analysis was carried out on an ACQUITY UPLC system using a BEH column of octadecyl silica gel (ODS-C18 silica gel, 1.7 µm) eluted with a gradient mixture of water containing 0.1% formic acid and acetonitrile containing 0.1 % formic acid (9:1 - 0:1), at a flow rate of 0.2 mL/min. The UPLC system was hyphenated to a Waters XEVO TQD triple quadruple mass spectrometer (Waters, Milford, USA).

3. Conclusion

The different fractions derived from *T. laxiflora* and *T. myriocarpa* leaf extracts contain constituents with DPPH scavenging, antibacterial, and antifungal capabilities. These capabilities could be further investigated by studying these fractions to evaluate their safety and efficacy *in-vivo*. This might lead to the introduction of safe natural antioxidant and antimicrobial drug candidates.

5. Conflicts of interest

The authors declare there are no conflicts of interest

6. Funding Sources

This work was funded by the National Research Centre, Egypt, under grant number 2/7/2.

7. Acknowledgment

The authors are thankful for the identification and authentication of the plant material by Ms. Therese Labib, the consultant taxonomist at Al-Orman and Al-Qubba Botanical Gardens.

8. References

[1] Zhang X-R, Kaunda JS, Zhu H-T, Wang D, Yang C-R, Zhang Y-J. The Genus *Terminalia* (Combretaceae): An Ethnopharmacological, Phytochemical and Pharmacological Review. Nat Prod Bioprospect 2019;9:357–92. https://doi.org/10.1007/s13659-019-00222-3.

[2] Wickens GE. Flora of Tropical East Africa, Combretaceae. East African Community, Royal Botanic Gardens, Kew, United Kingdom. Univ Of Chicago Press; 1973.

[3] Rajendran P, Nandakumar N, Rengarajan T, Palaniswami R, Gnanadhas EN, Lakshminarasaiah U, et al. Antioxidants and human diseases. Clinica Chimica Acta 2014;436:332–47. https://doi.org/10.1016/j.cca.2014.06.004.

[4] Patlevič P, Vašková J, Švorc P, Vaško L, Švorc P. Reactive oxygen species and antioxidant defense in human gastrointestinal diseases. Integr Med Res 2016;5:250–8. https://doi.org/10.1016/j.imr.2016.07.004.

[5] Muddathir AM, Yamauchi K, Mitsunaga T. Anti-acne activity of tannin-related compounds isolated from *Terminalia laxiflora*. Journal of Wood Science 2013;59:426–31. https://doi.org/10.1007/S10086-013-1344-4/TABLES/1.

[6] Elmalah A, Abdel khalik S, Abdelhady M, Taha K, Dawoud G. phytochemical composition and antioxidant activity of *Terminalia muelleri* and *Terminalia myriocarpa*. Egypt J Chem 2022;0:0–0. https://doi.org/10.21608/ejchem.2022.107513.4961.

[7] Gyawali R, Ibrahim SA. Natural products as antimicrobial agents. Food Control 2014;46:412–29. https://doi.org/10.1016/j.foodcont.2014.05.047.

[8] Mohieldin EAM, Muddathir AM, Yamauchi K, Mitsunaga T. Anti-caries activity of selected Sudanese medicinal plants with emphasis on *Terminalia laxiflora*. Revista Brasileira de Farmacognosia 2017;27:611–8. https://doi.org/10.1016/J.BJP.2017.04.002.

[9] Salih EYA, Kanninen M, Sipi M, Luukkanen O, Hiltunen R, Vuorela H, et al. Tannins, flavonoids and stilbenes in extracts of African savanna woodland trees *Terminalia brownii*, *Terminalia laxiflora* and *Anogeissus leiocarpus* showing promising antibacterial potential. South African Journal of Botany 2017;108:370–86. https://doi.org/10.1016/J.SAJB.2016.08.020.

[10] Salih EYA, Julkunen-Tiitto R, Lampi A-M, Kanninen M, Luukkanen O, Sipi M, et al. *Terminalia laxiflora* and *Terminalia brownii* contain a broad spectrum of antimycobacterial compounds including ellagitannins, ellagic acid derivatives, triterpenes, fatty acids and fatty alcohols. J Ethnopharmacol 2018;227:82–96. https://doi.org/https://doi.org/10.1016/j.jep.2018.04.030.

[11] Rashed K, Ono L. Hygeia :: journal for drugs and medicines OPEN ACCESS Phytochemical, Cytotoxic, anti-HSV-1 (Herpes Simplex Virus type-1) and anti bacterial studies of *Terminalia laxiflora* Engl. and Diels. HygeiaJDMedVol 2013;5:76–86.

[12] Boulis AG, El Zalabani SM, Ghaly NS, Sabry OM, El-Manawaty MA, Afifi AH, et al. Laxilignans A-C from the leaves of *Terminalia laxiflora* Engl. and their *α*-glucosidase inhibitory activity. Phytochem Lett 2024;59:79–86. https://doi.org/https://doi.org/10.1016/j.phytol.2023.12.005.

[13] Boulis AG, Zalabani SM El, Ghaly NS, Sabry OM, El-Manawaty MA, Afifi AH, et al. Secondary metabolites from the leaves of *Terminalia myriocarpa* and their *α*-glucosidase inhibitory potential. J Appl Pharm Sci 2024. https://doi.org/10.7324/JAPS.2024.170866.

[14] Chang Z, Zhang Q, Liang W, Zhou K, Jian P, She G, et al. A Comprehensive Review of the Structure Elucidation of Tannins from *Terminalia* Linn. Evid Based Complement Alternat Med 2019;2019:8623909. https://doi.org/10.1155/2019/8623909.

[15] Huang N, Siegel MM, Kruppa GH, Laukien FH. Automation of a Fourier transform ion cyclotron resonance mass spectrometer for acquisition, analysis, and e-mailing of high-resolution exact-mass electrospray ionization mass spectral data. J Am Soc Mass Spectrom 1999;10:1166–73. https://doi.org/10.1016/S1044-0305(99)00089-6.

[16] Kumar B, Singh A, Madhusudanan KP. Phytochemical Investigations of Genus *Terminalia*. Boca Raton: CRC Press; 2021. https://doi.org/10.1201/9781003181125.

[17] Cao J, Yin C, Qin Y, Cheng Z, Chen D. Approach to the study of flavone di- *C* -glycosides by high performance liquid chromatography-tandem ion trap mass spectrometry and its application to characterization of flavonoid composition in *Viola yedoensis*. Journal of Mass Spectrometry 2014;49:1010–24. https://doi.org/10.1002/jms.3413.

[18] Lee DY, Kim HW, Yang H, Sung SH. Hydrolyzable tannins from the fruits of *Terminalia chebula* Retz and their *α*-glucosidase inhibitory activities. Phytochemistry 2017;137:109–16. https://doi.org/10.1016/J.PHYTOCHEM.2017.02.006.

[19] Cao S, Brodie PJ, Callmander M, Randrianaivo R, Rakotobe E, Rasamison VE, et al. Saponins and a lignan derivative of *Terminalia tropophylla* from the Madagascar Dry Forest. Phytochemistry 2010;71:95–9. https://doi.org/10.1016/J.PHYTOCHEM.2009.09.027.

[20] Djoumbou-Feunang Y, Pon A, Karu N, Zheng J, Li C, Arndt D, et al. CFM-ID 3.0: Significantly Improved ESI-MS/MS Prediction and Compound Identification. Metabolites 2019;9. https://doi.org/10.3390/METABO9040072.

[21] Baldé MA, Tuenter E, Matheeussen A, Traoré MS, Cos P, Maes L, et al. Bioassay-guided isolation of antiplasmodial and antimicrobial constituents from the roots of *Terminalia albida*. J Ethnopharmacol 2021;267:113624. https://doi.org/10.1016/j.jep.2020.113624.

[22] Liu M, Katerere DR, Gray AI, Seidel V. Phytochemical and antifungal studies on *Terminalia mollis* and *Terminalia brachystemma*. Fitoterapia 2009;80:369–73. https://doi.org/10.1016/J.FITOTE.2009.05.006.

[23] Mallavarapu GR, Rao SB, Syamasundar K V. Chemical Constituents of the Bark of *Terminalia alata*. J Nat Prod 1986;49:549–50. https://doi.org/10.1021/np50045a039.

[24] Majumdar K, Biswas M, Som UK, Das S. Chemical constituents of the bark of *Terminalia myriocarpa*. Journal of Indian Chemical Society 2005;82:673–4.

[25] Marzouk MS, El-Toumy SA, Moharram FA, Shalaby NM, Ahmed AA. Pharmacologically active ellagitannins from *Terminalia myriocarpa*. Planta Med 2002;68:523–7. https://doi.org/10.1055/s-2002-32549.

[26] Lin TC, Hsu FL. Tannins and related compounds from *Terminalia arborea*. Chinese Pharmaceutical Journal 1996;48:167–75.

[27] Mopuri R, Ganjayi M, Banavathy KS, Parim BN, Meriga B. Evaluation of anti-obesity activities of ethanolic extract of *Terminalia paniculata* bark on high fat diet-induced obese rats. BMC Complement Altern Med 2015;15. https://doi.org/10.1186/S12906-015-0598-3.

[28] Wang W, Ali Z, Shen Y, Li XC, Khan IA. Ursane triterpenoids from the bark of *Terminalia arjuna*. Fitoterapia 2010;81:480–4. https://doi.org/10.1016/j.fitote.2010.01.006.
[29] Singh A, Bajpai V, Kumar S, Kumar B, Srivastava M, Rameshkumar KB. Comparative profiling of phenolic compounds from different plant parts of six *Terminalia* species by liquid chromatography–tandem mass spectrometry with chemometric analysis. Ind Crops Prod 2016;87:236–46. https://doi.org/10.1016/J.INDCROP.2016.04.048.
[30] Pfundstein B, El Desouky SK, Hull WE, Haubner R, Erben G, Owen RW. Polyphenolic compounds in the fruits of Egyptian medicinal plants (*Terminalia bellerica*, *Terminalia chebula* and *Terminalia horrida*): characterization, quantitation and determination of antioxidant capacities. Phytochemistry 2010;71:1132–48. https://doi.org/10.1016/j.phytochem.2010.03.018.
[31] Tsao S, Yin M. Antioxidative and Antiinflammatory Activities of Asiatic Acid, Glycyrrhizic Acid, and Oleanolic Acid in Human Bronchial Epithelial Cells. J Agric Food Chem 2015;63:3196–204. https://doi.org/10.1021/acs.jafc.5b00102.
[32] Hemalatha T, Pulavendran S, Balachandran C, Manohar BM, Puvanakrishnan R. Arjunolic acid: a novel phytomedicine with multifunctional therapeutic applications. Indian J Exp Biol 2010;48:238–47.
[33] Manosroi A, Jantrawut P, Ogihara E, Yamamoto A, Fukatsu M, Yasukawa K, et al. Biological Activities of Phenolic Compounds and Triterpenoids from the Galls of *Terminalia chebula*. Chem Biodivers 2013;10:1448–63. https://doi.org/10.1002/cbdv.201300149.
[34] Mkhwanazi BN, Serumula MR, Myburg RB, Van Heerden FR, Musabayane CT. Antioxidant effects of maslinic acid in livers, hearts and kidneys of streptozotocin-induced diabetic rats: effects on kidney function. Ren Fail 2014;36:419–31. https://doi.org/10.3109/0886022X.2013.867799.
[35] Yuan J-Q, Yang X-Z, Miao J-H, Tang C-P, Ke C-Q, Zhang J-B, et al. New Triterpene Glucosides from the Roots of *Rosa laevigata* Michx. Molecules 2008;13:2229–37. https://doi.org/10.3390/molecules13092229.
[36] Liu W, Liu T-C, Mong M. Antibacterial effects and action modes of asiatic acid. Biomedicine (Taipei) 2015;5:16. https://doi.org/10.7603/s40681-015-0016-7.
[37] Djoukeng JD, Abou-Mansour E, Tabacchi R, Tapondjou AL, Bouda H, Lontsi D. Antibacterial triterpenes from *Syzygium guineense* (Myrtaceae). J Ethnopharmacol 2005;101:283–6. https://doi.org/10.1016/j.jep.2005.05.008.
[38] Pu S, Ma Z, Wang Q. Anti-*Staphylococcus aureus* evaluation of gallic acid by isothermal microcalorimetry and principle component analysis. J Therm Anal Calorim 2019;136:1425–32. https://doi.org/10.1007/s10973-018-7726-5.
[39] Ahmed MD, Taher M, Maimusa AH, Rezali MF, Mahmud MIAM. Antimicrobial Activity of Methyl Gallate isolated from the Leaves of *Glochidion superbum* Against Hospital Isolates of Methicillin Resistant *Staphylococcus aureus*. Nps 2017;23:5–8. https://doi.org/10.20307/nps.2017.23.1.5.
[40] Blanco-Cabra N, Vega-Granados K, Moya-Andérico L, Vukomanovic M, Parra A, Álvarez de Cienfuegos L, et al. Novel Oleanolic and Maslinic Acid Derivatives as a Promising Treatment against Bacterial Biofilm in Nosocomial Infections: An in Vitro and in Vivo Study. ACS Infect Dis 2019;5. https://doi.org/10.1021/acsinfecdis.9b00125.
[41] Takó M, Kerekes EB, Zambrano C, Kotogán A, Papp T, Krisch J, et al. Plant Phenolics and Phenolic-Enriched Extracts as Antimicrobial Agents against Food-Contaminating Microorganisms. Antioxidants 2020;9:165. https://doi.org/10.3390/antiox9020165.
[42] Chung PY, Loh PLN, Neoh H, Ramli R. Alpha-amyrin as an anti-biofilm agent against methicillin-resistant and vancomycin-intermediate *Staphylococcus aureus*. Heliyon 2023;9:e17892. https://doi.org/10.1016/j.heliyon.2023.e17892.
[43] Adnan S-N-A, Ibrahim N, Yaacob WA. Transcriptome analysis of methicillin-resistant *Staphylococcus aureus* in response to stigmasterol and lupeol. J Glob Antimicrob Resist 2017;8:48–54. https://doi.org/10.1016/j.jgar.2016.10.006.
[44] Dordab T, Sourinejad I, Nazemi M. Antibacterial effect of the squalene extracted from the liver of the Persian Gulf spot tail shark *Carcharhinus sorrah* (Müller & Henle, 1839). Mdrsjrns 2021;10:251–8.
[45] Anwar R, Sukmasari S, Aisya L, Le F, Ilfani D, Y Y, et al. Antimicrobial Activity of *β*-Sitosterol Isolated from *Kalanchoe tomentosa* Leaves Against *Staphylococcus aureus* and *Klebsiella pneumonia*. Pakistan Journal of Biological Sciences 2022;25:602–7. https://doi.org/10.3923/pjbs.2022.602.607.
[46] Tintino S, Morais-Tintino C, Campina F, Pereira R, Costa M, Costa S, et al. Action of cholecalciferol and alpha-tocopherol on *Staphylococcus aureus* efflux pumps. EXCLI J 2016;15:315–22. https://doi.org/10.17179/excli2016-277.
[47] Togashi N, Shiraishi A, Nishizaka M, Matsuoka K, Endo K, Hamashima H, et al. Antibacterial Activity of Long-Chain Fatty Alcohols against *Staphylococcus aureus*. Molecules 2007;12:139–48. https://doi.org/10.3390/12020139.
[48] Inoue Y, Hada T, Shiraishi A, Hirose K, Hamashima H, Kobayashi S. Biphasic Effects of Geranylgeraniol, Teprenone, and Phytol on the Growth of *Staphylococcus aureus*. Antimicrob Agents Chemother 2005;49:1770–4. https://doi.org/10.1128/AAC.49.5.1770-1774.2005.
[49] Sri Charan Bindu B, Mishra DP, Narayan B. Inhibition of virulence of *Staphylococcus aureus* – a food borne pathogen – by squalene, a functional lipid. J Funct Foods 2015;18:224–34. https://doi.org/10.1016/j.jff.2015.07.008.
[50] Tian Q, Wei S, Su H, Zheng S, Xu S, Liu M, et al. Bactericidal activity of gallic acid against multi-drug resistance *Escherichia coli*. Microb Pathog 2022;173:105824. https://doi.org/10.1016/j.micpath.2022.105824.

[51] Li N, Luo M, Fu Y, Zu Y, Wang W, Zhang L, et al. Effect of Corilagin on Membrane Permeability of *Escherichia coli* , *Staphylococcus aureus* and *Candida albicans*. Phytotherapy Research 2013;27:1517–23. https://doi.org/10.1002/ptr.4891.

[52] Penduka D, Gasa N, Hlongwane M, Mosa R, Osunsanmi F, Opoku A. The antibacterial activities of some plant-derived triterpenes. African Journal of Traditional, Complementary and Alternative Medicines 2015;12:180. https://doi.org/10.4314/ajtcam.v12i6.19.

[53] Lin T-H, Wu C-C, Tseng C-Y, Fang J-H, Lin C-T. Effects of gallic acid on capsular polysaccharide biosynthesis in *Klebsiella pneumoniae*. Journal of Microbiology, Immunology and Infection 2022;55:1255–62. https://doi.org/10.1016/j.jmii.2021.07.002.

[54] Wang Y, Lu C, Zhao X, Wang D, Liu Y, Sun S. Antifungal activity and potential mechanism of Asiatic acid alone and in combination with fluconazole against *Candida albicans*. Biomedicine & Pharmacotherapy 2021;139:111568. https://doi.org/10.1016/j.biopha.2021.111568.

[55] Karygianni L, Cecere M, Argyropoulou A, Hellwig E, Skaltsounis AL, Wittmer A, et al. Compounds from *Olea europaea* and *Pistacia lentiscus* inhibit oral microbial growth. BMC Complement Altern Med 2019;19:51. https://doi.org/10.1186/s12906-019-2461-4.

[56] Haraguchi H, Kataoka S, Okamoto S, Hanafi M, Shibata K. Antimicrobial triterpenes from *Ilex integra* and the mechanism of antifungal action. Phytotherapy Research 1999;13:151–6. https://doi.org/10.1002/(SICI)1099-1573(199903)13:2<151::AID-PTR391>3.0.CO;2-C.

[57] Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride–methanol. J Lipid Res 1964;5:600–8. https://doi.org/10.1016/S0022-2275(20)40190-7.

[58] Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 1995;28:25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.

[59] Mensor LL, Menezes FS, Leitão GG, Reis AS, Santos TC dos, Coube CS, et al. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Research 2001;15:127–30. https://doi.org/10.1002/ptr.687.

[60] Das DC, De D, Bhattacharya S, Das M. Antibacterial activity and phytochemical analysis of *Cardanthera difformis* druce leaf extracts from West Bengal, India. International Journal of Phytomedicine 2013;5:446–51.

[61] Abd El-Hameed RH, Sayed AI, Mahmoud Ali S, Mosa MA, Khoder ZM, Fatahala SS. Synthesis of novel pyrroles and fused pyrroles as antifungal and antibacterial agents. J Enzyme Inhib Med Chem 2021;36:2183–98. https://doi.org/10.1080/14756366.2021.1984904.