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Profiling metabolites of *Ficus natalensis* hochst. fruit by UPLC-MS/MS and evaluation of anti-inflammatory activity

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Ficus natalensis **(Natal fig), an evergreen tree of the family Moraceae, is widely distributed across tropical and temperate regions and cultivated in Egypt. Members of the Moraceae family are traditionally employed as expectorants, hypoglycemic agents, and mild laxatives, with reported neuroprotective, analgesic, anti-inflammatory, and antihypertensive effects. The present study aimed to profile the secondary metabolites of *F. natalensis* fruit using ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) and test its anti-inflammatory potential. A total of 160 metabolites were annotated in both positive and negative ionization modes, belonging to diverse phytochemical classes such as phenolics (41), flavonoids (21), acids (26), glycosides (11), terpenoids, coumarins (4), iridoids (3), fatty acids/ester (22), sterols (8), sugar derivatives (6), and terpenoids (18). The anti-inflammatory activity of *F. natalensis* fruit methanolic extract was investigated using nitric acid (NO) inhibition assay, revealing an IC_{50} of 28.54 ± 1.66 µg/mL, compared to resveratrol as a standard anti-inflammatory with an IC_{50} value of 10.21 ± 0.68 µg/mL. Major bioactive constituents, including ellagic acid, gallic acid, betulinic acid, and quercetin derivatives, were identified and may underline the observed anti-inflammatory effect. The current findings highlight *F. natalensis* fruit as a rich source of bioactive metabolites with potential anti-inflammatory effect.**

Keywords Moraceae, *Ficus natalensis*, UHPLC-MS/MS, Anti-inflammatory activity

Plants are considered the cornerstone of human nutrition and medicine, serving as vital sources of bioactive compounds with high efficacy and safety. Unlike synthetic drugs, which often produce undesirable side effects, plant-derived metabolites such as alkaloids, glycosides, terpenoids, and flavonoids offer diverse therapeutic benefits and play crucial roles in maintaining human health[1].

The Moraceae family, commonly known as the fig or mulberry family, encompasses flowering plants of considerable biological and therapeutic significance. Members of this family exhibit a wide spectrum of pharmacological activities, including anti-inflammatory, anticancer, antimicrobial, and gastroprotective effects[2]. Beyond their medicinal roles, Moraceae species are widely utilized in the food, cosmetic, pharmaceutical, and agricultural industries. The family comprises approximately 38 genera and 1,100 species distributed across tropical and subtropical regions, with *Ficus* representing the largest genus, containing more than 850 species of trees, shrubs, and vines[3]. The genus *Ficus* holds exceptional nutritional and medicinal importance, providing edible fruits and phytochemically rich plant parts[4]. They originated in tropical habitat types, with about 100 species originating in African countries and nearby islands. Moreover, these species have been used in traditional medical practices as hypoglycaemic, laxative, anti-rheumatic, anthelmintic, and antihypertensive agents, and also for inflammation, dyspepsia, stomach-aches, and dysentery[5,6].

The Natal fig (*Ficus natalensis* Hochst.) is an evergreen species cultivated in Egypt primarily for ornamental and environmental purposes. Beyond its landscape value, the species is increasingly recognized as a rich reservoir of phytochemicals, including flavonoids, tannins, and triterpenoid saponins, which contribute to its therapeutic potential[3]. Previous biologically guided studies on *F. natalensis* leaves have demonstrated potent antioxidant, antimicrobial, cytotoxic, and anti-inflammatory activities[12,13].

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In recent years, metabolomics tools are widely applied for phytochemical profiling of natural products[14]. Among these tools, UPLC-MS/MS technique is well suited for profiling of non-volatile metabolites among plant samples including fruits and vegetables[15]. LC-MS/MS was applied for profiling of several *Ficus* species including *F. deltoidei*, *F. drupacea* and *F. sycomorus*[16], *F. benghalensis* aerial root[17], and *F. lyrata* leaves and fruits[18].

The main goal of the current study was to profile the secondary metabolites of *F. natalensis* fruits using UPLC-MS/MS and to correlate the resulting chemical profile with its anti-inflammatory activity.

Results and discussion

Metabolites profiling of *F. natalensis* fruit extract using UPLC-MS/MS in both positive and negative ionization modes (Fig. 1) led to the identification of 160 metabolites belonging to different phytochemical classes, including phenolics (41), flavonoids (21), acids (26), glycosides (11), terpenoids, coumarins (4), iridoids (3), fatty acids/ester (22), sterols (8), sugar derivatives (6), and terpenoids (18) (Fig. 2). Compound identification was achieved by comparing their molecular ions ($[M\text{-}H]^-$ or $[M+H]^+$), retention times (Rt), characteristic fragment ions (Figure S1-S6), and reference spectra from the literature. The chemical structures of selected phytochemicals identified in *F. natalensis* fruit extract are illustrated in Fig. 3.

Phenolic compounds

UPLC-MS/MS profiling of *F. natalensis* fruit revealed 41 phenolic metabolites, belonging to hydroxybenzoic and hydroxycinnamic acids, galloyl derivatives, lignans, and complex caffeoylquinic esters. Several bioactive compounds such as, gallic acid (peak 96; m/z 169.0134 $[M\text{-}H]^-$ $C_7H_6O_5^-$) and ellagic acid (peak 116; m/z 301.0064 $[M\text{-}H]^-$ $C_{14}H_6O_8^-$) were identified. Caffeic acid (m/z 179.0341 $[M\text{-}H]^-$ $C_9H_8O_4^-$) and syringic acid (m/z 193.050 $[M\text{-}H]^-$ $C_9H_{10}O_5^-$), were annotated. Such phenolic compounds are known for potent antioxidant and anti-inflammatory properties via scavenging reactive oxygen species and modulating the NF-κB signaling pathway[19]. The presence of gallic and ellagic acids, common in the Moraceae family, in accordance with previous reports on *F. carica* and *F. religiosa*[20]. Moreover, gallic acid, methyl gallate, syringic acid, and ellagic acid were reported in *F. carica* fruits[21], with several biological properties including antioxidant, antidiabetic, and antimicrobial[22]. Phenolic compounds such as rosmarinic acid-di-*O*-hexoside (peak 110, m/z 683.1801 $[M\text{-}H]^-$), dicaffeoylquinic acid (peak 117, m/z 515.1169 $[M\text{-}H]^-$), and 1-*O*-caffeoyl-3-*O*-sinapoylquinic acid (peak 117, m/z 515.1169 $[M\text{-}H]^-$) were detected in *F. natalensis* fruits. Such results are consistent with the rich content of phenolic compounds in *F. benghalensis* and *F. religiosa*[23]. The caffeoylquinic derivatives are known to enhance antioxidant capacity and modulate inflammatory pathways[24]. The phenolic compounds of *F. natalensis* fruit are structurally varied compared to the previously reported for other *Ficus* fruits, which revealed their potent antioxidant, anti-inflammatory, and therapeutic potential. The detection of oleuropein aglycone, a phenolic compound abundantly found in olive fruit with myriad of biological activities[25], further valorizes the phytochemical and pharmacological significance of *F. natalensis* fruit. The detection of several hydroxycinnamic and hydroxybenzoic acids in *F. natalensis* aligns with reports of widespread distribution of these metabolites in

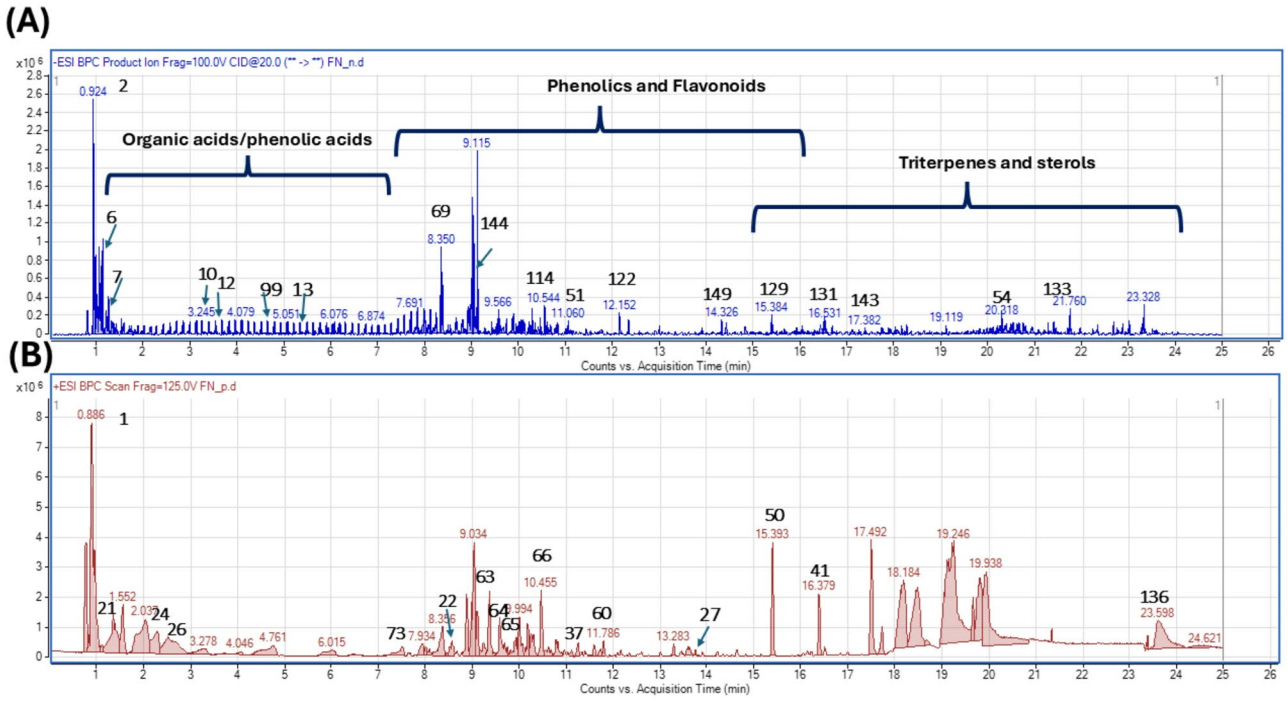

Fig. 1. UPLC-MS base peak chromatograms of *Ficus natalensis* fruits **A**) BPC in negative mode **B**) BPC in positive mode. Peaks are numbered according to Table 1, corresponding to putatively annotated metabolites.

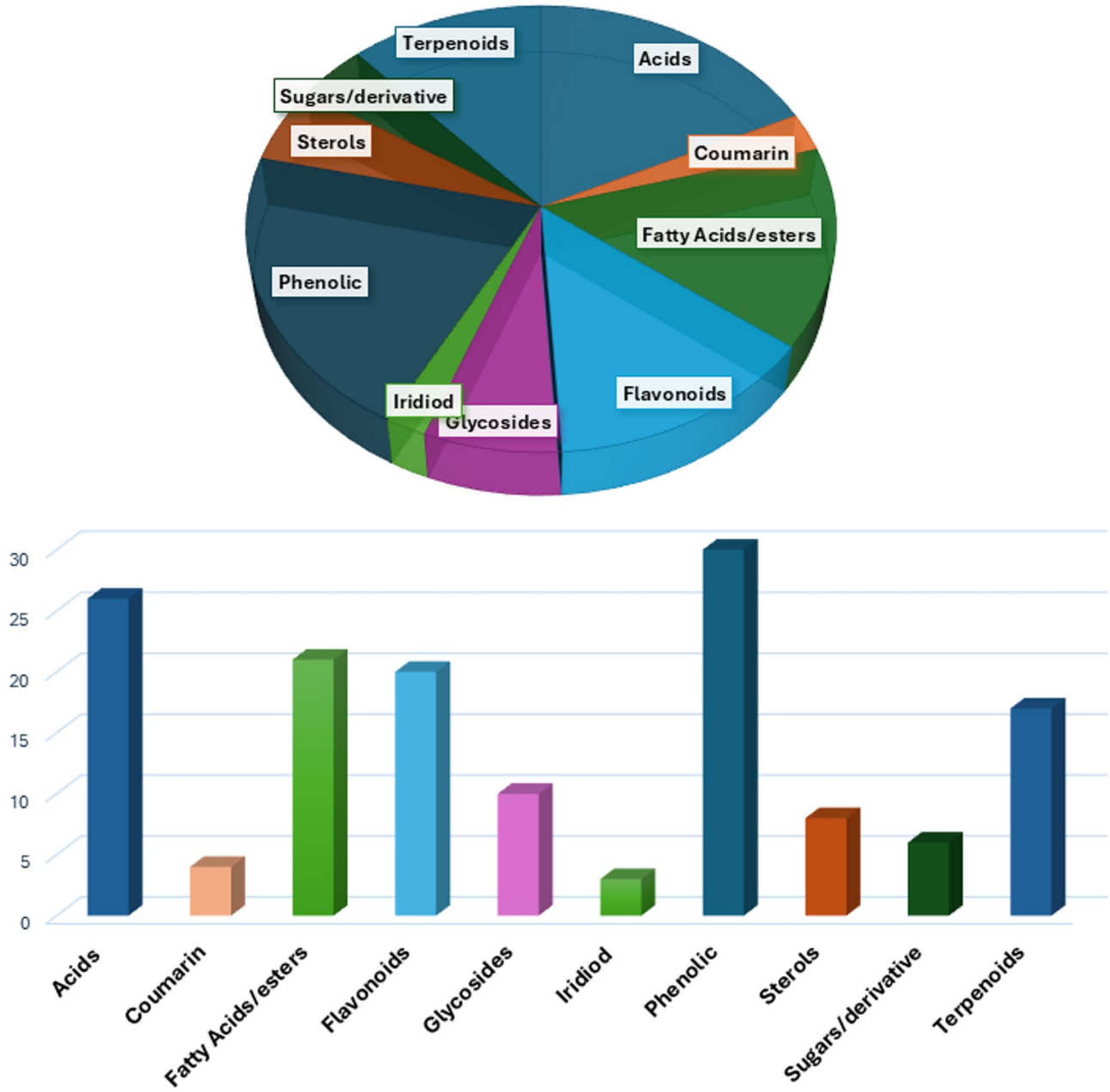

Fig. 2. Representative bar-chart and pie chart of identified metabolites classes in *F. natalensis* fruits using UPLC-MS negative and positive modes based on number of compounds identified in each class.

the Ficus genus[26]. In accordance with previous reports, phenolic compounds were enriched in *F. deltoidei*, *F. drupacea*, and *F. sycomorus*[16].

Flavonoids

Flavonoids are a diverse group of natural polyphenolic compounds widely distributed in vegetables, fruits, and grains[27]. As major plant secondary metabolites, they play pivotal roles in human health as they constitute one of the most significant groups of bioactive phytochemicals in human diet and with potential antioxidant, antimicrobial, anti-inflammatory, and cytoprotective effects[27]. A total of 21 flavonoids were annotated in *F. natalensis* fruit with both aglycones and glycosidic derivatives. In the negative ionization mode, quercetin (peak 60; m/z 301.0340 $[M\text{-}H]^-$) and its glycosides such as quercetin-3-*O*-glucoside (isoquercitrin) (peak 58; m/z 463.089 $[M\text{-}H]^-$) were identified. Similarly, kaempferol-dihexoside (peak 57; m/z 609.1433 $[M\text{-}H]^-$) exhibited a characteristic neutral loss of 308 amu, corresponding to the sequential elimination of two hexose moieties, thereby confirming its di-glycosylated structure[28]. Quercetin, kaempferol, and isorhamnetin glycosides such as isoquercetin, avicularin, and quercetin-3-*O*-pentosylhexoside were identified. The detection of these flavonoids agrees with earlier reports on *F. carica*, *F. sycomorus*, and *F. benghalensis*, where these flavonols dominated

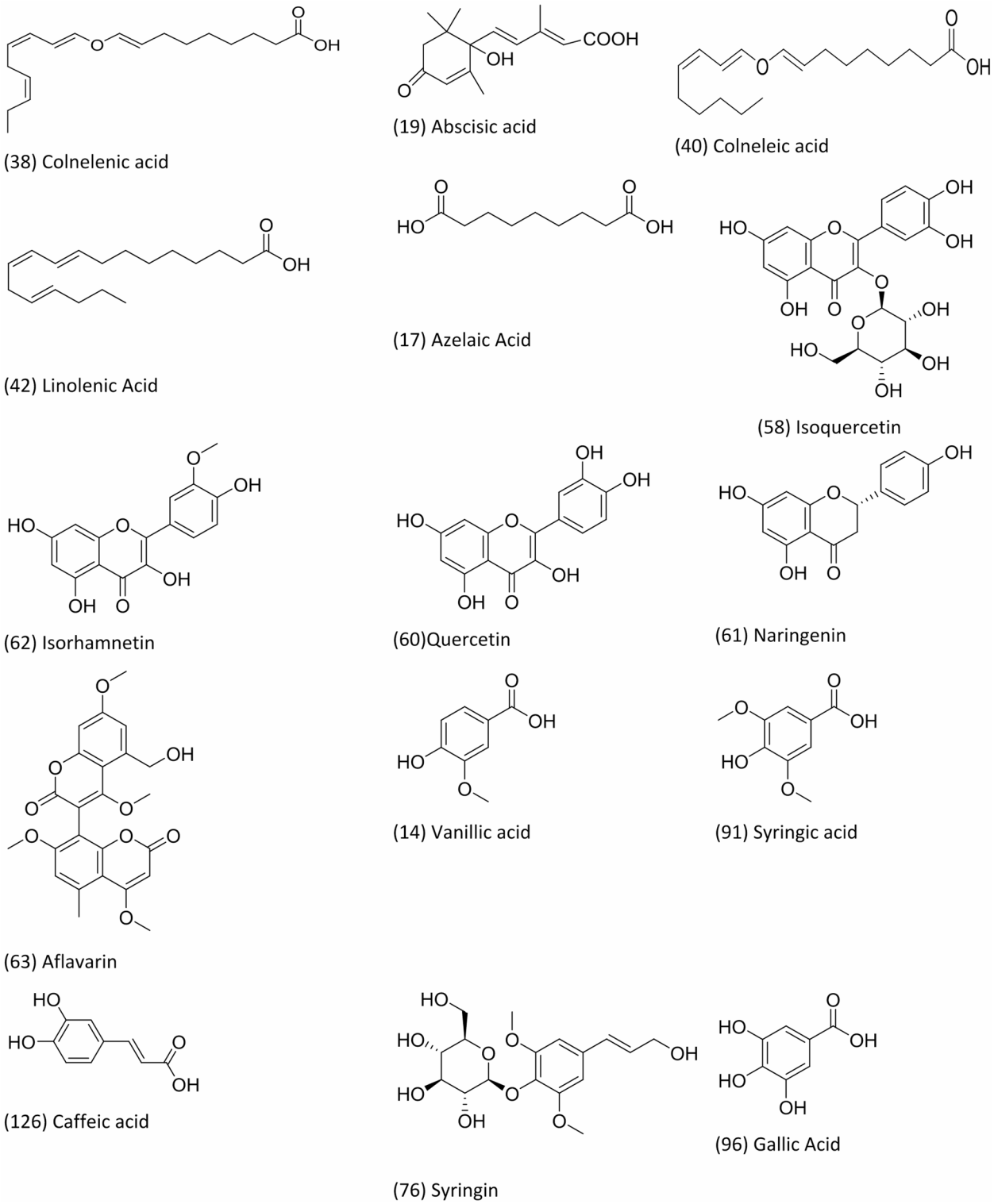

Fig. 3. The chemical structure of selected phytochemicals identified in *F. natalensis* fruit extract using UPLC-MS/MS analysis, the name and number of the compounds as listed in Table 1.

and were linked to strong antioxidant and anti-inflammatory activities[26]. The diversity of flavonoid glycosides identified here highlights the metabolic richness of *F. natalensis* and underscores its potential as a dietary source of bioactive flavonoids. In accordance with previous studies, LC-MS/MS was applied for profiling of three *Ficus* species including *F. deltoidei*, *F. drupacea* and *F. sycomorus*[16] revealing the annotation of 90 metabolites belonging to phenolic acids, flavonoids, furanocoumarins, fatty acids and terpenoids[16]. UPLC-MS profiling of *F. benghalensis* aerial root revealed identification of 84 metabolites belonging to isoflavonoids, triterpenes,

No	R_T	Mass (MW)	m/z $[M-H]^-$ m/z $[M+H]^+$	Formala	Cpd name	Class	Fragmentation	Error	References
Acids									
1.	0.889	226.068	225.0607	$C_7H_{14}O_8^-$	Glucoheptonic acid	Acid	215,165,135, 89	0.86	
2.	0.902	166.0471	165.0398	$C_5H_{10}O_6^-$	Arabonic acid	Acid	133	0.65	
3.	1.075	354.0936	353.0863	$C_{16}H_{18}O_9^-$	3-O-Caffeoyl quinic acid	Acid	191	1.5	[30]
4.	1.214	476.1355	475.128	$C_{16}H_{28}O_{16}^-$	Malic acid dihexoside	Acid	133	2.28	[67]
5.	1.258	192.0626	191.0553	$C_7H_{12}O_6^-$	Quinic acid	Acid	133, 93	0.79	[68]
6.	1.312	134.021	133.0137	$C_4H_6O_5^-$	Malic acid	Acid	115, 71	0.55	[69]
7.	1.477	192.0263	191.019	$C_6H_8O_7^-$	Citric acid	Acid	111, 87, 76, 57	0.72	[69]
8.	1.5	112.0155	111.0083	$C_5H_4O_3^-$	Furoic acid	Acid	-	0.51	[70]
9.	1.809	148.0365	147.0292	$C_5H_8O_5^-$	Citramalic acid	Acid	113	0.67	[37]
10.	3.284	132.0416	131.0344	$C_5H_8O_4^-$	Dimethyl malonate	Acid	130, 91, 70	0.62	[37]
11.	3.482	330.094	329.0868	$C_{14}H_{18}O_9^-$	Vanillic acid-4-O-β-D-glucopyranoside	Acid	167, 122	1.1	[71]
12.	3.65	206.0419	205.0346	$C_7H_{10}O_7^-$	Homocitric acid	Acid	153, 111	0.78	[37]
13.	5.315	316.0779	315.0707	$C_{13}H_{16}O_9^-$	Dihydroxybenzoic acid hexoside I	Acid	153, 109	1.51	[72]
14.	5.733	168.0415	167.0342	$C_8H_8O_4^-$	Vanillic acid	Acid	122, 108	0.75	[71]
15.	7.591	138.0308	137.0235	$C_7H_6O_3^-$	P-Hydroxybenzoic acid	Acid	137, 93	0.89	[73]
16.	7.92	176.0676	175.0604	$C_7H_{12}O_5^-$	2-Isopropylmalic acid	Acid	137, 115, 113	0.84	[71]
17.	10.982	188.104	187.0968	$C_9H_{16}O_4^-$	Azelaic Acid	Acid	169, 125, 97	0.83	[74]
18.	11.295	194.0572	193.05	$C_{10}H_{10}O_4^-$	Ferulic acid	Acid	149	0.68	[25]
19.	11.781	264.1351	263.1278	$C_{15}H_{20}O_4^-$	Abscisic acid	Acid	145, 117	1.09	[37]
20.	11.817	202.1197	201.1124	$C_{10}H_{18}O_4^-$	Sebacic Acid	Acid	183, 139	0.85	[75]
21.	12.6	216.1352	215.1279	$C_{11}H_{20}O_4^-$	Undecanedioic Acid	Acid	197, 153	1	[76]
22.	8.638	374.1187	375.1263	$C_{16}H_{22}O_{10}^+$	Geniposidic acid	Acid	282, 207, 159	2.57	[77]
23.	1.103	364.0983	365.1056	$C_{14}H_{20}O_{11}^+$	2,3,4,5-Tetra-O-acetylhexonic acid	Acid	176, 157, 118, 101	2.25	[78]
24.	2.164	156.04	157.047	$C_7H_8O_4^+$	Heptadienedioic acid	Acid	132, 108	2.49	[79]
25.	2.65	140.009	141.0158	$C_6H_4O_4^+$	Coumalic acid	Acid	141, 101	2.45	
26.	2.69	118.026	119.0337	$C_4H_6O_4^+$	Succinic Acid	Acid	119, 101	0.17	
Coumarins									
27.	13.919	262.048	263.0561	$C_{13}H_{10}O_6^+$	Maclurin	Coumarin	223, 100	0.09	[37]
28.	1.257	380.072	381.0799	$C_{17}H_{16}O_{10}^+$	5-O-β-Dglucopyranosyl-6- hydroxyangelicin	Coumarin	381, 219, 201	2.17	[80]
29.	8.78	338.0987	337.0915	$C_{16}H_{18}O_8^-$	P-Coumaroylquinic acid	Coumarin	163, 191	1.46	[81]
30.	14.1	386.1345	385.1273	$C_{21}H_{22}O_7^-$	Pteryxin	Coumarin	238, 112	2.03	
Fatty acids									
31.	13.322	288.2284	287.2211	$C_{16}H_{32}O_4^-$	Dihydroxyhexadecanoic acid	Fatty acid	287, 269	1.66	[82]
32.	13.704	328.2232	327.216	$C_{18}H_{32}O_5^-$	9,12,13-Trihydroxy-10,15- octadecadienoic acid	Fatty acid	309, 291, 239, 229, 221, 211, 191, 183, 171, 137	1.81	[82]
33.	13.969	308.1972	307.1899	$C_{18}H_{28}O_4^-$	16-Hydroxy-9-oxo-10E,12E,14E-octadecatrienoic acid	Fatty acid	289, 235, 211, 209, 185, 121	1.58	[83]
34.	14.005	330.239	329.2317	$C_{18}H_{34}O_5^-$	9,12,13-Trihydroxy-10-octadecenoic acid	Fatty acid	229, 211, 171	1.61	[82]
35.	15.17	310.2128	309.2055	$C_{18}H_{30}O_4^-$	Unknown Fatty acid	Fatty acid	238, 112	1.61	
36.	11.08	414.259	415.2663	$C_{22}H_{38}O_7^+$	Ascorbyl palmitate	Fatty acid	371, 115	2.73	[84]
37.	11.336	228.134	229.1411	$C_{12}H_{20}O_4^+$	Dodecenedioic acid	Fatty acid	183, 165	2.4	[82]
38.	12.624	292.204	293.2107	$C_{18}H_{28}O_3^+$	Colnelenic acid	Fatty acid	195, 100	0.39	[37]
39.	12.973	212.1402	213.146	$C_{12}H_{20}O_3^+$	Dihydrojasmonic acid	Fatty acid	195, 100	2.48	[37]
40.	13.047	294.2188	295.2262	$C_{18}H_{30}O_3^+$	Colneleic acid	Fatty acid	195, 100	0.76	[37]
41.	16.173	276.209	277.2161	$C_{18}H_{28}O_2^+$	Stearidonic Acid	Fatty acid	235, 221, 207	-0.05	[14]
42.	16.798	278.2246	279.2319	$C_{18}H_{30}O_2^+$	Linolenic Acid	Fatty acid	144, 137	-0.05	[85]
43.	16.766	296.2339	295.2265	$C_{18}H_{32}O_3^-$	13-Hydroxyoctadeca-9,15-dienoic acid	Fatty acid	295, 277, 183	1.25	[82]
44.	17.532	298.2495	297.2423	$C_{18}H_{34}O_3^-$	Ricinoleic acid	Fatty acid	194	1.3	[37]
45.	18.086	300.2652	299.2579	$C_{18}H_{36}O_3^-$	2-Hydroxyethyl palmitate	Fatty acid	238, 155, 112	1.24	
46.	18.967	272.2341	271.2268	$C_{16}H_{32}O_3^-$	12-hydroxy-13-methyltetradecanoate	Fatty acid	248, 155, 112	1.01	[86]
47.	20.331	328.2965	327.2892	$C_{20}H_{40}O_3^-$	2-Hydroxybutyl palmitate	Fatty acid	144, 100	1.26	
48.	22.146	400.3534	399.346	$C_{24}H_{48}O_4^-$	Monoheneicosanoin	Fatty acid	355, 316, 125, 100	1.83	
49.	22.205	356.3278	355.3205	$C_{22}H_{44}O_3^-$	2-Hydroxydocosanoic acid	Fatty acid	316, 125, 100	1.24	
50.	15.392	352.2617	353.2692	$C_{21}H_{36}O_4^+$	Glyceryl linolenate	Fatty acid	304, 221,100	-0.35	[37]
Continued									

No	R_T	Mass (MW)	m/z [M-H]⁻ m/z [M + H]⁺	Formala	Cpd name	Class	Fragmentation	Error	References
51.	11.112	392.2756	391.2683	$C_{20}H_{40}O_7^-$	D-Glucitol monomyristate	Fatty acid ester	187, 137	1.81	
52.	20.093	314.2809	313.2736	$C_{19}H_{38}O_3^-$	Methyl 2-hydroxystearate	Fatty acid ester	239, 125, 100	1.24	
Flavonoids									
53.	11.729	550.1662	549.1589	$C_{26}H_{30}O_{13}^-$	Liquiritin apioside	Flavanoids	255, 135	2.44	87
54.	20.31	356.1641	355.1572	$C_{21}H_{24}O_5^-$	Unknown flavanoid	Flavanoids	327, 255, 144	-1.68	
55.	15.656	370.1398	369.1325	$C_{21}H_{22}O_6^-$	Sophoraisoflavanone A	Flavanoids	369,208, 161, 135	1.83	88
56.	9.803	596.135	595.1278	$C_{26}H_{28}O_{16}^-$	Quercetin-3-O-pentosylhexoside	Flavanoids	300, 271, 179	2.78	89
57.	10.115	610.1505	609.1433	$C_{27}H_{30}O_{16}^-$	Kaempferol dihexoside	Flavanoids	447, 285, 197, 153	2.84	28
58.	10.278	464.0937	463.0864	$C_{21}H_{20}O_{12}^-$	Isoquercetin	Flavanoids	301, 271, 255	1.74	90
59.	10.752	484.025	483.0178	$C_{22}H_{12}O_{13}^-$	Methyl (S)-flavogallonate	Flavanoids	450, 407, 299	2.79	91
60.	11.885	302.0413	301.034	$C_{15}H_{10}O_7^-$	Quercetin	Flavanoids	153, 181	1.33	92
61.	12.541	272.0673	271.06	$C_{15}H_{12}O_5^-$	Naringenin	Flavanoids	151, 177	1.21	93
62.	12.853	316.057	315.0497	$C_{16}H_{12}O_7^-$	Isorhamnetin	Flavanoids	300	1.3	94
63.	9.3	454.1268	455.1341	$C_{24}H_{22}O_9^+$	Aflavarin	Flavanoids	281, 195	-0.43	37
64.	9.748	404.1649	405.1736	$C_{25}H_{24}O_5^+$	Osajin	Flavanoids	319, 279, 251, 223, 137	-2.56	95
65.	9.834	492.1243	493.1315	$C_{23}H_{24}O_{12}^+$	Tricin O-hexoside	Flavanoids	331	2.46	96
66.	10.556	434.085	435.0923	$C_{20}H_{18}O_{11}^+$	Avicularin	Flavanoids	301	-0.08	97
67.	12.207	248.1022	249.1095	$C_{14}H_{16}O_4^+$	Prenyl caffeate	Flavanoids	226, 100	2.65	37
68.	8.233	666.1763	665.1687	$C_{30}H_{34}O_{17}^-$	Chrysoeriol-O-acetylglucoside-O-glucoside	Flavanoids	443, 353, 315, 285	3.3	98
69.	8.372	708.1872	707.1801	$C_{32}H_{36}O_{18}^-$	Kalambroside A	Flavanoids	443, 353, 285	2.99	99
70.	8.58	452.223	451.2159	$C_{27}H_{32}O_6^-$	Kushenol D	Flavanoids	443, 353, 234, 183	-3.13	88
71.	8.97	696.1881	695.1812	$C_{31}H_{36}O_{18}^-$	Apigenin C-pentoside-C- hydroxyferuloyl-pentoside	Flavanoids	353, 341	2.03	100
72.	3.789	414.0925	415.1003	$C_{21}H_{18}O_9^+$	Flavan-3-ol-(4 – 2)- phloroglucinol	Flavanoids	289, 139, 121	2.59	101
73.	7.39	436.1557	437.1628	$C_{25}H_{24}O_7^+$	Artonin J	Flavanoids	429, 370, 311, 226	-3.53	102
Glycosides									
74.	3.828	324.082	325.0894	$C_{15}H_{16}O_8^+$	Umbelliferone glucoside	Glycosides	280, 163, 119, 107	2.48	47
75.	8.657	414.1499	415.1573	$C_{19}H_{26}O_{10}^+$	Ptelatoside A	Glycosides	371, 238	2.66	
76.	10.474	372.1419	373.1494	$C_{17}H_{24}O_9^+$	Syringin	Glycosides	371,209	0.08	48
77.	8.094	444.1977	443.1905	$C_{21}H_{32}O_{10}^-$	Cynaroside A	Glycosides	443, 353	1.8	103
78.	9.289	416.1667	415.1591	$C_{19}H_{28}O_{10}^-$	Phenethyl primeveroside	Glycosides	337, 223, 175, 151	1.57	28
79.	10.185	442.1826	441.1752	$C_{21}H_{30}O_{10}^-$	(1′S, 6′R)-8’-Hydroxyabscisic acid β-D-glucoside	Glycosides	415, 371	1.33	104
80.	10.462	472.1925	471.1851	$C_{22}H_{32}O_{11}^-$	Eugenol rutinoside	Glycosides	163, 148	1.96	49
81.	10.748	394.1817	393.1746	$C_{17}H_{30}O_{10}^-$	3-Hexenyl b-primeveroside	Glycosides	250, 99, 57	2.24	105
82.	10.783	416.2028	415.1956	$C_{20}H_{32}O_9^+$	Ethyl 7-epi-12-hydroxyjasmonate glucoside	Glycosides	389/405	1.81	106
83.	10.869	420.1395	419.1322	$C_{21}H_{24}O_9^-$	Rhapontigenin O-glucoside	Glycosides	401, 299, 281, 257, 241, 225	2.58	107
84.	11.273	402.1498	401.1433	$C_{18}H_{26}O_{10}^-$	Benzyl beta-primeveroside	Glycosides	289, 269, 193	2.76	108
Iridoids									
85.	1.692	434.1403	435.1477	$C_{18}H_{26}O_{12}^+$	8-O-Acetylshanzhiside	Iridiod	365, 229, 182	2.12	43
86.	1.051	696.2087	695.2019	$C_{28}H_{40}O_{20}^-$	Monotropein dihexoside	Iridoid glycosides	533, 383, 353, 191, 133	2.61	45
87.	2.816	452.1504	451.1433	$C_{18}H_{28}O_{13}^-$	Shanzhiside methyl ester	Iridoid glycosides	243, 101	2.6	44
Phenolics									
88.	1.186	214.0453	215.0526	$C_9H_{10}O_6^+$	2-Hydroxyethyl gallate	Phenolic	176, 141	2.41	37
89.	1.993	164.0474	165.0546	$C_9H_8O_3^+$	3’-Hydroxycinnamic acid	Phenolic	132, 119	-0.05	
90.	3.749	332.1076	333.1154	$C_{14}H_{20}O_9^+$	Hydroxybenzoic acid derivative	Phenolic	275,165, 141, 121	3.16	109
91.	7.919	198.0504	199.0577	$C_9H_{10}O_5^+$	Syringic acid	Phenolic	141, 97	2.38	37
92.	8.464	242.0402	243.0475	$C_{10}H_{10}O_7^+$	3-Galloyl-glycerinaldehyd	Phenolic	218, 169	2.47	37
93.	9.698	438.1503	439.1574	$C_{21}H_{26}O_{10}^+$	Fortuneanoside B	Phenolic	409, 207	2.3	37
94.	11.043	240.061	241.0683	$C_{11}H_{12}O_6^+$	Lignicol	Phenolic	227, 187	2.4	37
95.	11.221	378.1289	379.1362	$C_{19}H_{22}O_8^+$	Oleuropein aglycone	Phenolic	294, 207, 100	2.52	37
96.	2.322	170.0207	169.0134	$C_7H_6O_5^-$	Gallic Acid	Phenolic	125	0.81	110
97.	2.727	524.1143	523.1072	$C_{23}H_{24}O_{14}^-$	3,5-di-O-(3-O-methyl galloyl) quinic acid	Phenolic	191, 169	2.31	111
Continued									

No	R_T	Mass (MW)	m/z $[M-H]^-$ m/z $[M+H]^+$	Formala	Cpd name	Class	Fragmentation	Error	References
98.	2.929	512.1145	511.1073	$C_{22}H_{24}O_{14}^-$	Galloyl-syringic acid-hexoside I	Phenolic	313, 196, 125	2.08	112
99.	4.925	302.0987	301.0914	$C_{13}H_{18}O_8^-$	Tachioside	Phenolic	179, 161,139, 121	1.5	113
100.	5.72	154.0259	153.0186	$C_7H_6O_4^-$	Dihydroxybenzoic acid II	Phenolic	109	0.72	71
101.	6.696	302.0622	301.0549	$C_{12}H_{14}O_9^-$	Pyrogallol-2-O-glucuronide	Phenolic	175, 168, 140	1.58	114
102.	7.422	342.094	341.0867	$C_{15}H_{18}O_9^-$	Caffeic acid-O-glucoside	Phenolic	179, 135	1.1	115
103.	7.595	708.1864	707.1791	$C_{39}H_{32}O_{13}^-$	Caffeoylquinic acid dimer	Phenolic	353, 191	-2.06	116
104.	8.111	474.1349	473.1275	$C_{20}H_{26}O_{13}^-$	Caffeic acid pentosyl hexoside	Phenolic	443, 353, 341, 179	2.44	117
105.	8.187	316.1143	315.1069	$C_{14}H_{20}O_8^-$	vanilloloside	Phenolic	281, 137	1.56	118,119
106.	8.411	516.1458	515.1379	$C_{22}H_{28}O_{14}^-$	3-O-(β-Dglucopyranosyl)-caffeoyl quinic acid	Phenolic	353, 285, 173	2.16	109,120
107.	8.535	184.0363	183.029	$C_8H_8O_5^-$	Methyl gallate	Phenolic	167, 124	0.9	37
108.	8.632	386.0831	385.0758	$C_{16}H_{18}O_{11}^-$	O-Feruloylgalactaric acid I	Phenolic	353, 183	1.83	121
109.	8.954	526.1296	525.1223	$C_{23}H_{26}O_{14}^-$	Galloyl-eudesmic acid-hexoside I	Phenolic	353, 341, 183, 168	2.68	112
110.	9.007	684.1872	683.18	$C_{30}H_{36}O_{18}^-$	Rosmarinic acid-di-O-hexoside	Phenolic	521, 359, 341	2.95	122
111.	9.109	326.0987	325.0914	$C_{15}H_{18}O_8^-$	4-O-Glucosylcinnamic acid	Phenolic	293, 163	1.45	123
112.	9.308	448.1563	447.1491	$C_{19}H_{28}O_{12}^-$	Apigenin derivative	Phenolic	401, 293, 269, 161, 101	1.76	124
113.	9.95	306.0363	305.0291	$C_{14}H_{10}O_8^-$	Methyl brevifolincarboxylate	Phenolic	273,245,221,217,187,000	1.23	125
114.	9.987	462.172	461.1647	$C_{20}H_{30}O_{12}^-$	Rebouoside C	Phenolic	299, 137	1.76	126
115.	10.195	374.1561	373.1487	$C_{17}H_{26}O_9^-$	Decenedioyl quinic acid	Phenolic	199, 173, 125	1.59	
116.	10.317	302.0047	300.9974	$C_{14}H_6O_8^-$	Ellagic Acid	Phenolic	573, 483	1.57	37
117.	10.504	516.1241	515.1169	$C_{25}H_{24}O_{12}^-$	Dicaffeoyl-quinic acid	Phenolic	353, 335, 191, 179, 173, 161, 135	2.64	127
118.	10.724	448.0982	447.091	$C_{21}H_{20}O_{11}^-$	Quercetin 3-deoxyhexoside	Phenolic	303, 265	2.36	128
119.	11.316	560.1509	559.143	$C_{27}H_{28}O_{13}^-$	1-O-caffeoyl-3-O-sinapoylquinic acid	Phenolic	559, 381, 193	2.13	129
120.	12.034	210.0911	209.0842	$C_{11}H_{14}O_4^-$	Sinapyl alcohol	Phenolic	176, 112, 91	-1.92	130
121.	12.08	538.166	537.1588	$C_{25}H_{30}O_{13}^-$	Coumaroyl-dihydromonotropein	Phenolic	465, 238, 112, 91	2.66	131
122.	12.271	178.0618	177.0549	$C_{10}H_{10}O_3^-$	4-Methoxycinnamic acid	Phenolic	163, 149	1.19	37
123.	13.45	344.0514	343.0442	$C_{17}H_{12}O_8^-$	Tri-O-methylellagic acid	Phenolic	328, 312, 297, 285, 269	1.78	91
124.	9.159	292.0205	291.0133	$C_{13}H_8O_8^-$	Brevifolincarboxylic acid	Phenolic	243, 194	1.39	37
125.	9.241	368.1085	367.1015	$C_{17}H_{20}O_9^-$	3-Feruloylquinic acid	Phenolic	191, 173	2.26	132
126.	9.345	180.0414	179.0341	$C_9H_8O_4^-$	Caffeic acid	Phenolic	135, 134, 117, 107, 89	0.81	133
127.	9.699	336.0832	335.076	$C_{16}H_{16}O_8^-$	5-O-Caffeoylshikimic acid	Phenolic	179, 161, 135	1.31	133
128.	14.582	294.1817	293.1744	$C_{17}H_{26}O_4^-$	6-Gingerol	Phenolic	277, 221, 177	1.42	134
Sterols									
129.	15.432	578.2728	577.2659	$C_{30}H_{42}O_{11}^-$	Hellebrigenin glucoside	Sterols	415	-0.1	135
130.	15.935	392.2908	391.2834	$C_{24}H_{40}O_4^-$	Deoxycholic acid	Sterols	391, 337, 297, 279	1.85	136
131.	16.473	432.226	431.2188	$C_{28}H_{32}O_4^-$	Sterostrein A	Sterols	413, 387	4.11	37
132.	20.878	480.3795	479.3721	$C_{29}H_{52}O_5^-$	Unknown sterol	Sterols	381, 125, 100	1.99	
133.	21.573	462.3694	461.3623	$C_{29}H_{50}O_4^-$	Unknown sterol	Sterols	409, 144, 100	1.55	
134.	21.927	448.3534	447.3459	$C_{28}H_{48}O_4^-$	Typhasterol	Sterols	384, 125, 100	1.83	
135.	21.141	588.4212	589.4285	$C_{39}H_{56}O_4^+$	Stigmasteryl ferulate	Sterols	575, 487, 394	-3.35	37
136.	23.515	396.3755	397.3827	$C_{29}H_{48}^+$	Stigmasta-3,5-diene	Sterols	335, 144, 68	0.15	37
Sugar Derivatives									
137.	0.993	384.1256	383.1184	$C_{14}H_{24}O_{12}^-$	Acetyl-maltose	Sugar derivatives	357,365, 191	1.17	106
138.	1.034	534.1774	533.1702	$C_{19}H_{34}O_{17}^-$	Glucopyranosyl fructofuranosi de quinic acid	Sugar derivatives	383, 353, 191,	2.16	136
139.	2.016	162.0521	161.0448	$C_6H_{10}O_5^-$	Meglutol	Sugar derivatives	159, 141, 123	0.74	37
140.	8.771	294.1301	293.1228	$C_{12}H_{22}O_8^-$	2-O-glucosyl-hexanoic acid	Sugar derivatives	248, 183, 153	1.36	113
141.	9.779	382.1821	381.1748	$C_{16}H_{30}O_{10}^-$	Methylbutyl-O-pentosylhexoside	Sugar derivatives	337, 305	1.82	15
142.	1.331	342.1144	341.1072	$C_{12}H_{22}O_{11}^-$	Sucrose	Sugars	178, 160	1.77	137
Terpenoids									
143.	17.321	434.2413	433.2341	$C_{28}H_{34}O_4^-$	20-β-Hydroxyscutione	Terpenoid	238, 152, 112	4.39	138
Continued									

No	R_T	Mass (MW)	m/z [M-H]⁻ m/z [M+H]⁺	Formala	Cpd name	Class	Fragmentation	Error	References
144.	9.38	390.1511	389.1437	$C_{17}H_{26}O_{10}^-$	Loganin	Terpenoids	327, 267	1.53	[28]
145.	10.517	426.187	425.1799	$C_{21}H_{30}O_9^-$	Abscisic acid-hexose	Terpenoids	263, 153	1.94	[139]
146.	11.245	514.2394	513.2315	$C_{25}H_{38}O_{11}^-$	Taxchinin J	Terpenoids	323, 263, 221, 179, 161, 125	1.98	[140]
147.	11.436	538.3329	537.3256	$C_{33}H_{46}O_6^-$	unknown triterpenoid	Terpenoids	463, 389, 238, 91	-3.44	
148.	13.53	406.2694	405.2625	$C_{24}H_{38}O_5^-$	3α,12α-dihydroxy-7-oxo-5β-cholanic acid	Terpenoids	343, 361, 289	2.55	[141]
149.	14.239	408.2856	407.2783	$C_{24}H_{40}O_5^-$	Cholic acid	Terpenoids	311, 238, 112, 107	2	[142]
150.	14.84	488.3479	487.3406	$C_{30}H_{48}O_5^-$	Asiatic Acid	Terpenoids	453, 417	2.24	[37]
151.	15.586	266.1536	265.1468	$C_{15}H_{22}O_4^-$	Deoxyartemisinin	Terpenoids	213, 100	-1.79	[37]
152.	16.679	556.2886	555.2816	$C_{28}H_{44}O_{11}^-$	Forskoditerpenoside C	Terpenoids	521, 485	-0.22	[143]
153.	19.403	456.3585	455.3512	$C_{30}H_{48}O_3^-$	Betulinic acid	Terpenoids	327, 317, 353,409, 437	1.89	[144]
154.	20.763	472.3534	471.3462	$C_{30}H_{48}O_4^-$	Maslinic acid	Terpenoids	423, 393, 405	1.85	[145]
155.	22.494	608.4261	607.4188	$C_{35}H_{60}O_8^-$	Sporminarin B	Terpenoids	544, 355, 316, 125, 100	2.72	
156.	22.674	620.4261	619.4188	$C_{36}H_{60}O_8^-$	Fasciculic acid A	Terpenoids	581, 545, 527	2.71	[37]
157.	8.918	388.1707	389.1779	$C_{18}H_{28}O_9^+$	Tuberonic acid	Terpenoids	207, 163, 89	2.64	[133]
158.	10.743	378.1652	379.1727	$C_{20}H_{26}O_7^+$	Cnicin	Terpenoids	247, 229, 211, 155, 129	2.6	[146]
159.	14.816	510.3319	511.339	$C_{32}H_{46}O_5^+$	Ganodermic acid TQ	Terpenoids	453, 365, 239, 100	2.65	[37]
160.	14.829	452.3287	453.3361	$C_{30}H_{44}O_3^+$	Ganoderic acid SZ	Terpenoids	351, 262	0.3	[37]

Table 1. Chemical Metabolites tentatively identified in *F. natalensis* fruits by UPLC-MS/MS at negative and positive modes.

sterols, fatty acids, cyclic peptides, apocarotenoids, hydroxybenzoates, and hydroxycinnamates[17]. The metabolite profiles of *F. lyrata* leaves and fruits grown in Egypt were performed using UPLC-MS/MS with identification of 72 metabolites belonging to flavonoids, phenolic acids and fatty acids[18].

Organic and phenolic acids

A total of 26 organic acids were identified in *F. natalensis* fruit by UPLC-MS/MS, comprising aliphatic, hydroxy, dicarboxylic, and phenolic acids. Citric acid (peak 7; m/z 191.0190 $[M-H]^-$), malic (peak 6; m/z 133.0137 $[M-H]^-$), succinic (peak 26; m/z 119.0337 $[M+H]^+$), and quinic acid (peak 5; m/z 191.0553 $[M-H]^-$) were identified. Moreover, abscisic (peak 19) and geniposidic acids (peak 22) were detected. The organic acid profile of *F. natalensis* fruit revealed by UPLC-MS/MS aligns closely with those reported for other Ficus species[16]. The predominance of citric, malic, and quinic acids play a pivotal role in regulation of fruit acidity and flavor as previously reported in *F. carica* and *F. sycomorus*[29]. However, *F. natalensis* showed a richer diversity of dicarboxylic acids, including azelaic and sebacic acids, which are less commonly reported in *Ficus* fruits. Phenolic acids including 3-*O*-caffeoylquinic (peak 3; m/z 353.063 $[M-H]^-$)[30], vanillic (peak 14; m/z 167.0342 $[M-H]^-$), p-hydroxybenzoic (peak 15; m/z 137.0235$[M-H]^-$), and ferulic acids (peak 18; m/z 193.0561$[M-H]^-$), together with their glycosides were identified. Phenolic acids are well known for their biological importance including antioxidant and anti-inflammatory activities[31]. The detection of phenolic acids such as caffeoylquinic, ferulic, and vanillic acids is consistent with earlier findings in *F. benghalensis* and *F. racemosa*[32].

Coumarins

Three coumarin derivatives were identified in *F. natalensis* fruit, including maclurin (peak 27; m/z 263.0561 $[M+H]^+$), pteryxin (peak 29; m/z 385.127 $[M-H]^-$), and 5-*O*-β-D-glucopyranosyl-6-hydroxyangelicin (peak 28; m/z 381.0799 $[M+H]^+$) which was characterized by a diagnostic aglycone ion at m/z 219 after sugar loss. Coumarins are known for their antithrombotic, hepatoprotective, and anti-inflammatory activities mediated through suppression of prostaglandin synthesis[33]. These compounds are frequently reported in *Ficus* spp. and have been associated with potential health effects[34]. Their occurrence supports the ethnopharmacological relevance of *F. natalensis*, particularly in traditional remedies for infections and vascular disorders. The detection of *p*-coumaroylquinic acid (m/z 337.0915 $[M-H]^-$) further emphasizes the coumarin-phenolic interface typical of *Ficus* species[35]. In accordance with previous studies, furanocoumarins were identified in *F. deltoidei*, *F. drupacea* and *F. sycomorus*[16].

Fatty acids and esters

Several fatty acids were identified in *F. natalensis* using UPLC-MS/MS including linoleic, palmitic, and oleic acids. A diverse profile of 22 fatty acids and related esters was detected in *F. natalensis* fruit, encompassing hydroxylated, polyunsaturated, and conjugated species such as linolenic acid, stearidonic acid, ricinoleic acid, colnelenic acid, and dihydrojasmonic acid. Hydroxylated fatty acids including 13-hydroxyoctadeca-9,15-dienoic acid and dihydroxyhexadecanoic acid are known oxylipin intermediates were also identified. Unsaturated fatty acids such as linolenic acid (m/z 279.2319 $[M+H]^+$) and stearidonic Acid (m/z 277.2161 $[M+H]^+$) were

detected. Linolenic and stearidonic acids are ω−3 polyunsaturated fatty acids (PUFAs) that act as precursors for oxylipins, a group of lipid mediators with potent anti-inflammatory and cardioprotective effects[36]. Colneleic (peak 40; m/z 295.2262 $[M+H]^+$) and colnelenic acids (peak 38; m/z 293.2107 $[M+H]^+$)[37], are oxygenated linoleic acid derivatives were detected in *F. natalensis* fruit. Similarly, the detection of 9,12,13-trihydroxy-10,15-octadecadienoic acid and 16-hydroxy-9-oxo-10,12,14-octadecatrienoic acid indicates lipid oxidation processes yielding trihydroxy and oxo-fatty acids, compounds frequently associated with oxidative stress responses and anti-inflammatory activities. Similar lipid constituents have been reported in other Ficus fruits including *F. carica* and *F. sycomorus*, supporting their nutritional value and cardioprotective potential[38,39]. Fatty acid esters such as ascorbyl palmitate (peak 36; m/z 415.2663 $[M+H]^+$) and glyceryl linolenate (peak 50; m/z 353.2692 $M+H]^+$) were identified further expands the lipid profile of *F. natalensis*. Ascorbyl palmitate, an esterified form of vitamin C and palmitic acid, acts as a potent lipid-soluble antioxidant[40]. Glyceryl linolenate is a monoacylglycerol derivative previously reported in *F. exasperata* with potential anti-inflammatory activity[41].

Iridoids and glycosides

Iridoids, a class of bioactive metabolites widely distributed in plant species with myriad of biological properties including neuroprotective, hepatoprotective, anti-inflammatory, antitumor, hypoglycemic, and hypolipidemic activities[42]. Three iridoid glycosides were identified including 8-*O*-acetylshanzhiside (peak 85; m/z 435.1477 $[M+H]^+$; $C_{18}H_{26}O_{12}^+$)[43] and shanzhiside methyl ester (peak 87; m/z 451.1433 $[M-H]^-$)[44]. Their fragmentation patterns, including neutral losses of glucose and acetyl moieties, confirmed their structures. Monotropein derivatives (peak 86; m/z 695.2019 $[M-H]^-$) showed sequential sugar losses (−162, −180), typical of di-glucosylated iridoids[45]. These metabolites are known for anti-inflammatory and hepatoprotective properties[46].

A total of 11 glycosides were identified, including coumarin, phenolic, monoterpenoid, and lignan-derived sugar conjugates. Glycosides were primarily represented by iridoid, phenolic, and terpenoid-linked sugars. Umbelliferone hexoside (peak 74; m/z 325.0894 $[M+H]^+$) exhibited characteristic fragmentation at m/z 163, 119 corresponding to umbelliferone aglycone[47]. Syringin (peak 76; m/z 373.1494 $[M+H]^+$)[48] and eugenol rutinoside (peak 80; m/z 471.1851 $[M-H]^-$)[49], were identified based on the loss of sugar moieties (162 amu for hexose and 308 amu for rutinoside). Syringin is a phenylpropanoid glycoside, is widely distributed in various plant species with potential health effects including immunomodulatory, anti-tumor, antihyperglycemic, and antihyperlipidemic effects[50].

Terpenoids and sterols

A diverse array of terpenoids including 18 compounds were detected in *F. natalensis* fruit, including abscisic acid-hexose, betulinic acid, maslinic acid, asiatic acid, and ganoderic derivatives. Betulinic acid (peak 153; m/z 455.3512 $[M-H]^-$) and maslinic acid (peak 154; m/z 471.3462 $[M-H]^-$) were detected confirmed the presence of lupane- and oleanane-type triterpenes. Asiatic acid (peak 150; m/z 487.3406 $[M-H]^-$) and cholic acid (peak 149; m/z 407.2783 $[M-H]^-$) were also observed. The detection of forskoditerpenoside C (peak 152; m/z 555.2816 $[M-H]^-$) and taxchinin J (peak 146; m/z 513.2315 $[M-H]^-$) suggests the coexistence of diterpenoid glycosides. Pentacyclic triterpenoids such as betulinic, maslinic, and asiatic acids are widespread in Moraceae and are well known for their antioxidant, anti-inflammatory, and cytoprotective activities[51]. Eight sterols were identified in *F. natalensis* fruit, including hellebrigenin glucoside, deoxycholic acid, sterostrein A, typhasterol, stigmasteryl ferulate, and stigmasta-3,5-diene. These compounds reflect the plant's diverse sterol biosynthetic machinery, contributing to membrane stability and signaling. The occurrence of phytosterols such as stigmasteryl ferulate and stigmasta-3,5-diene agrees with reports in *F. carica*, *F. sycomorus*, and *F. benghalensis* fruits[52]. Stigmasteryl ferulate, a conjugated steryl ester, is known for potent antioxidant and anti-inflammatory activities, while typhasterol, a brassinosteroid intermediate, plays crucial roles in plant growth and stress tolerance. The sterol profile of *F. natalensis* fruit underscores its potential bioactivity as cardioprotective, hypocholesterolemic, and anti-inflammatory health effects, consistent with the reported bioactivity of sterol-enriched Ficus extracts[53].

Sugar derivatives

Six sugar-related metabolites were annotated in *F. natalensis* fruit, including common disaccharides (sucrose and acetyl-maltose) and conjugated sugar acids such as glucopyranosyl fructofuranoside quinic acid, 2-*O*-glucosyl-hexanoic acid, and methylbutyl-O-pentosylhexoside. Sucrose was previously reported in *F. carica* and *F. sycomorus* fruits, where it contributes to sweetness and osmo-protection during ripening[54].

GNPS molecular networking

GNPS molecular networking organized the detected metabolites into structurally related clusters, enabling annotation propagation and revealing both known compounds and structurally related unknown analogues. The presence of hub nodes and cluster-specific fragmentation patterns supports the reliability of putative annotations and highlights the chemical diversity of *F. natalensis* fruit metabolites (Figure S7- S9). Cluster-based annotation of the GNPS molecular network suggested that the metabolome of *F. natalensis* fruit is dominated by flavonoid glycosides and their acylated derivatives, alongside triterpenoid saponins and diverse phenolic constituents.

Anti-inflammatory activity

Inflammation is a complex biological defence mechanism initiated by the immune system in response to harmful stimuli such as microbial infections, toxins, physical injury, and chemical irritants[55]. However, when inflammation becomes chronic or excessive, it contributes to the pathogenesis of several degenerative and metabolic disorders, including cancer, Alzheimer's disease, atherosclerosis, and cardiovascular diseases[37]. Nitric oxide (NO) plays a pivotal role in the inflammatory process, as secreted by endothelial cells, macrophages, and neurons to serve as a vital chemical mediator in immune responses[56]. Under pathological conditions,

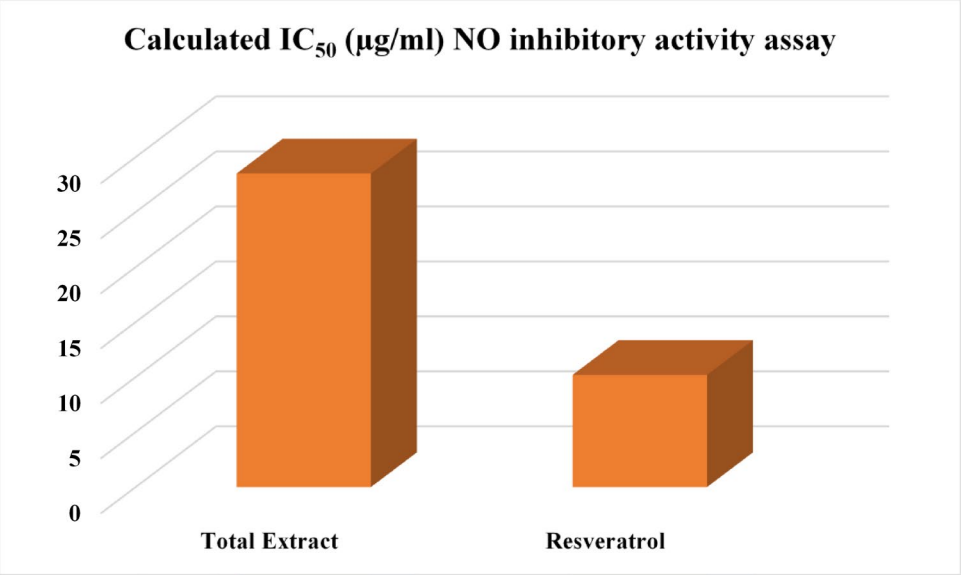

Fig. 4. Calculated IC_{50} (µg/ml) of *F. natalensis* fruits methanolic extract of and Resveratrol by NO inhibitory activity assay.

overproduction of NO contributes to oxidative stress and tissue injury. Therefore, inhibition of excessive NO production has become a target mechanism for evaluating the anti-inflammatory potential of therapeutic agents[57]. Recently, natural products derived from medicinal plants have drawn significant attention as potential sources of anti-inflammatory agents. Various phytochemicals such as phenolics, flavonoids, terpenoids, steroids, and saponins have been reported to exert potent anti-inflammatory activities through multiple mechanisms including inhibition of NO production[58].

A nitric oxide inhibitory assay was utilized to investigate the anti-inflammatory properties of the methanolic extract from *F. natalensis* fruit. The extract revealed significant anti-inflammatory activity with an IC_{50} value of 28.54 ± 1.66 µg/mL, compared to resveratrol as a reference anti-inflammatory with IC_{50} of 10.21 ± 0.68 µg/mL (Fig. 4). The diverse phytochemical profile of *F. natalensis* notably phenolic acids, flavonoids, terpenoids, and coumarins collectively contribute to the extract's anti-inflammatory potential. Potent antioxidant, anti-inflammatory, and antibacterial qualities are well-known for several identified metabolites, especially gallic acid and its derivatives[59]. For instance, gallic acid reduces inflammation by preventing the release of cytokines that promote inflammation[60]. Furthermore, substances like betulinic acid, ellagic acid, and quercetin greatly enhance the extract's bioactivity[37,61]. These findings are consistent with previous studies on other Ficus species, such as *F. benghalensis*, *F. carica*, and *F. religiosa*, which also exhibited significant anti-inflammatory effects[62–64]. Furthermore, *F. natalensis* leaves exhibited a potent anti-inflammatory potential when tested in vitro and in vivo[65]. These results highlight the therapeutic potential of *F. natalensis* fruit extract as a natural source of anti-inflammatory agents. Recent studies have proved several pharmacological effects of Ficus species[7]. For instance, the ethanol extract of *F. racemosa* leaf showed anti-inflammatory activity by inhibiting the formation of prostaglandins PGE2 and PGD2, 5-lipoxygenase (IC_{50} = 58 µg/ml), and decreased the activity of COX-1 (IC_{50} = 83 µg/ml)[7]. *F. natalensis* leaf extract can attenuate $CdCl_2$-induced testicular impairments by inhibiting oxidative cell damage and inflammation[11].

Conclusion

The present study provides a comprehensive phytochemical profiling of *Ficus natalensis* fruit using UPLC-MS/MS, alongside an evaluation of its anti-inflammatory potential. A total of 160 metabolites were tentatively annotated, representing diverse phytochemical classes, with phenolics and flavonoids being the predominant constituents. The fruit extract demonstrated notable anti-inflammatory activity (IC_{50} = 28.54 µg/mL) compared to resveratrol (IC_{50} = 10.21 µg/mL), supporting its potential as a source of bioactive compounds. These findings highlight the chemical richness of *F. natalensis* fruit and underscore its potential value as a natural source of pharmacologically relevant metabolites. However, metabolite identification in this study was based on accurate mass measurements and MS/MS fragmentation data and thus remains putative (MSI Level 2) in the absence of validation using authentic reference standards. In addition, isomeric compounds cannot be excluded due to similarities in fragmentation patterns. While UPLC-HRMS is highly effective for qualitative metabolite profiling, definitive structural elucidation and absolute quantification require complementary techniques such as co-analysis with authentic standards and nuclear magnetic resonance (NMR) spectroscopy. Furthermore, although molecular networking and spectral similarity approaches supported metabolite annotation, more advanced metabolomics workflows, including feature-based molecular networking and extensive spectral library matching, would further enhance annotation confidence. From a biological perspective, the current study focused on anti-inflammatory activity; therefore, additional investigations encompassing antioxidant,

antimicrobial, enzyme inhibitory, and cytotoxic assays, as well as in vivo studies, are warranted to fully elucidate the pharmacological potential and underlying mechanisms of action of *F. natalensis* fruit. Overall, this work provides a valuable foundation for future phytochemical and pharmacological studies, while emphasizing the need for further validation and comprehensive biological evaluation to substantiate the therapeutic potential of this species.

Materials and methods

Plant material

The fruits of *F. natalensis* Hochst. were collected from the Horticultural Research Institute in Giza, Egypt in October 2024. A voucher specimen with the number "ERU-PH-01-Fn/2024", has been deposited at the Pharmacy Department of the Faculty of Pharmacy, Egyptian Russian University. The plant was botanically verified by Dr. Rim Hamdy, Department of Plant Taxonomy, Faculty of Science, Cairo University.

Chemicals

MilliQ water supplied by a Millipore MR3 purifier system was used for UPLC analysis. Acetonitrile (J. T. Baker, Deventer, The Netherlands, LC-MS grade, purity ≥ 99.5%) and formic acid (J. T. Baker, Deventer, The Netherlands, LC-MS grade, purity ≥ 99%). All chemicals and standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Samples extraction and preparation for UPLC-HRMS/MS analysis

Freeze-dried *F. natalensis* fruits ($n = 3$ each) as biological replicates were lyophilized and ground in liquid nitrogen using a pestle and mortar. The extraction procedure was conducted as previously described in[25]. About 100 mg of the powdered samples was mixed with 6 mL 100% methanol containing 10 µg/mL umbelliferone as an internal standard (Sigma-Aldrich, St. Louis, MO, USA, purity ≥ 98.0%) and homogenized with an Ultra-Turrax (IKA, Staufen, Germany) at 11,000 rpm, 5 × 60 s with 1 min break intervals. Extracts were then vortexed for 1 min, centrifuged at 3000 g for 10 min, and filtered through a 22 µm pore size filter.

UPLC-HRMS/MS nalysis

Chromatographic separation of the metabolites was performed using a previously validated method[25]. An Acquity Waters ultra-performance liquid chromatography (UPLC) system (Waters Corp., Milford, MA, USA) equipped with an HSS T3 column (100 × 1.0 mm, particle size 1.8 µm; Waters Corp.) was employed. A gradient elution system was followed at a flow rate of 150 µL/min, starting from formic acid in water (0.1%): CH_3CN = 95:5–100% CH_3CN within 35 min, then isocratically for a further 10 min; flow rate 70 µL/min. The electrospray ionization (ESI) source was operated under the following parameters: electrospray voltage, 4.0 kV; capillary temperature, 275 °C; sheath gas, N_2.

Metabolite annotation

HRMS data were processed using MS Hunter software for peak alignment, molecular feature extraction, molecular formula prediction, and MS/MS fragment analysis, owing to its robust handling of high-resolution mass data and reliable qualitative annotation capabilities. Metabolites were putatively characterized based on accurate mass measurements, retention behaviour, isotopic pattern matching, and MS/MS fragmentation patterns, through comparison with the reference literature. Metabolite annotation levels were assigned in accordance with the guidelines of the Metabolomics Standards Initiative (MSI). Isotopic pattern similarity was incorporated during formula prediction using MS Hunter, and annotations were accepted only when the isotopic pattern fit exceeded 85% and the mass error was below 10 ppm. Signals exhibiting mass deviations greater than 10 ppm were manually re-evaluated and excluded when confidence was insufficient.

GNPS feature-based molecular MS/MS network

Using the Feature-based Molecular Networking (FBMN) workflow (version release_28.2)[18] on GNPS, a molecular network was created. The resulting aligned list of features were exported in a mgf file besides their feature quantification table in csv format. The values of feature quantification table were uploaded onto the FBMN page of GNPS. MS^2 spectra were filtered, all MS/MS fragment ions within ± 17 Da of the precursor *m/z*were removed, and only the top 6 fragment ions in the ± 50 Da window through the spectrum were utilized. The precursor and fragment ion masses were both set to 0.02 Da. Edges of the molecular network were filtered to have a cosine score above 0.7 and more than 5 matched peaks between the connected nodes. The edges between two nodes were kept in the network if and only if each of the nodes appeared in each other's respective top 10 most similar nodes. The size of clusters in the network was set to a maximum of 100.The molecular networks were visualized using Cytoscape 3.9.1[17]. Metabolites were annotated based on molecular formula and their fragmentation pattern, compared to earlier reported data aided with public literatures, libraries, and databases.

Extraction for biological investigation

About 200 g of dried powdered *F. natalensis* fruits were macerated in methanol at room temperature with stirring. The process was carried out three times until exhaustion. The compiled methanol extract was concentrated under vacuum using Rotary evaporator to yield about 15 g of dried methanol extract.

Anti-inflammatory activity

NO inhibition activity of the tested samples was determined by using a sodium nitroprusside (SNP) as previously reported[66]. The Greiss reagent was used to detect the nitrite ions that are produced when the NO radical produced by SNP in an aqueous solution at physiological pH combines with oxygen. For 150 min, the reaction mixture

(2 mL) in phosphate-buffered saline (PBS; pH 7.4) with different concentrations of the tested samples and SNP (10 mM) was incubated at 25 °C. Following the incubation period, 1 mL of the reaction mixture samples was diluted with 1 mL of Greiss reagent, which consists of 0.1% naphthyl ethylene diamine dihydrochloride and 1% sulphanilamide (w/v) in 5% phosphoric acid (v/v). The mixture was incubated at 25 °C and for 30 min. The absorbance of these solutions was measured at 546 nm against the blank solution (without SNP). Resveratrol was used as a reference standard. All the tests were performed in triplicate. The percent inhibition activity was calculated using the formula.

$$\text{Inhibition } \% = \left[\frac{\text{A control} - \text{A sample}}{\text{A control}}\right] \text{x100} \quad (1)$$

where, $A_{control}$ is the absorbance of the control reaction at 546 nm and A_{test} represents the absorbance of a test reaction at the same wavelength. Tested material concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against concentration.

Statistical analysis

The results of biological investigation were analysed in triplicate and displayed as average ± standard deviation of the mean (SD).

Data availability

All data generated or analysed during this study are included in this published article.

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Author contributions

EMS; Data curation, Investigation, Writing original manuscript, **RH**; plant identification and collection, Investigation. **MHB**; Conceptualization, Supervision, Data curation, Investigation, Writing-review & editing.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval

The plant was botanically identified by Prof. Dr. Rim Hamdy, Botany Department, Faculty of Science, Cairo University, Egypt. A voucher specimen has been deposited at Pharmacognosy Department, Faculty of Pharmacy, Egyptian Russian university, Egypt No = ERU-PH-01-Fn/2024.

Plant ethics

The methods in plant collection and experimentation were carried out in accordance with the guidelines prescribed by the American Society of Plant Taxonomists and adopted by the institutional research committee. The plant with collected with permission from the Botany Department, Faculty of Science, Cairo University, Egypt, and the Horticultural Research Institute in Giza, Egypt.

Additional information

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