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Larvicidal and Cytotoxic Activity of *Cordyline terminalis* Kunth and its Metabolites Profiling via UPLC-MS/MS

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Abstract: Mosquitoes are the foremost arthropod carriers of infection. As a rule, *Culex pipiens*, known as a house mosquito, could be a vector of many viruses and infectious diseases. The aqueous methanolic extract of *Cordyline terminalis* Kunth. leaves (syn. *Cordyline fruticose*) was examined against *Culex pipiens* Linnaeus (Diptera: Culicidae) to detect its insecticidal activity. Assessing *Cordyline terminalis* Kunth larvicidal activity against *C. pipiens* 3rd instar larvae at 24 and 48 hours after application. The results displayed larval mortality after 24-h and 48-h, with LC_{50} values of 268.96 and 248.43 ppm, respectively. Aqueous methanolic extract and its fractions (petroleum ether, chloroform, ethyl-acetate, and *n*-butanol) have been demonstrated to exhibit an inhibitory effect against the breast cancer cell line (MCF)-7, liver cancer cell line (HepG)-2, and colon cancer cell line (HCT)-116. The aqueous methanolic extract displayed the most potent cytotoxic activity against the tested cell lines, with IC_{50} of 9.02, 10.9, and 11.13 µg/mL, respectively. The cytotoxic activity of petroleum ether exhibited IC_{50} of 9.28, 11.4, and 11.85 µg/mL, respectively. A total of 76 compounds were tentatively identified in *Cordyline terminalis* Kunth leaves extract including 30 flavonoids, 13 carboxylic acids, 7 phenolic acids, 4 steroidal saponins and other different identified compounds, in both negative and positive ion modes by using UPLC. Overall, the results indicate that the *Cordyline terminalis* extract has notable and promising larvicidal and cytotoxic activity which may refer to steroidal saponins and anthocyanins that were detected in the plant.

Keywords: *Cordyline*; insecticidal; cytotoxic; breast; colon; liver; LC-MS/MS

1. INTRODUCTION

Mosquitoes are known to transmit a variety of human diseases. *Culex pipiens* is widespread in Egypt, endangering humans and transmitting many diseases [1]. It is a symptom of yellow fever[2], Rift Valley fever virus[3], West Nile virus[4], fluriasis[5] and other common diseases worldwide, causing morbidity and death in humans and animals, as well as some economic loss in many countries, including costs of health care. Protocols for mosquito control refer to the use of synthetic insecticidal agents such as larvicidal or adult repellent agents[6]. Although synthetic insecticides have bad side effects on the environment and health[7], many natural plants have been used as a source of medicinal factors for centuries and are still used for treating various illnesses, infections, and insecticidal mosquitoes. At present, there are a remarkable number of drugs that have been isolated from many plant species. UPLC-ESI-QTOF-MS/MS improves the identification and characterization of various compounds using their molecular weight and fragmentation of their MS/MS [8,9]. Moreover, UPLC-ESI-QTOF-MS/MS provides fragmentation and separation of many ions, which helps with the structural identification and isomer characterization [8-10]. *Cordyline terminalis* Kunth (syn. *Cordyline fruticosa* A. Chev) belongs to the family Asparagaceae, which is distributed in tropical and subtropical regions [11,12]. The genus *Cordyline* contains 15 branched trees and shrubs [(13)]. *Cordyline* plants are reported to be used in the treatment of bloody cough, dysentery, high fever, kidney disorders, aches, toothache, and constipation. *Cordyline terminalis* is one of the most important ornamental plants, with numerous cultivars. Its leaves have green, red, or purple foliage, despite its variety of medicinal uses. It refers to tropical Asia,

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Australia, Bali, and the Pacific Islands. Recently it was cultivated as an ornamental plant in Egypt and all over the world [(14)]. *Cordyline terminalis* is known to display multiple pharmacological actions, such as antioxidant, antipyretic, analgesic, anti-proliferative bioactivity, and antibacterial activities [15,16]. Leaves were also used as anti-inflammatory agents, and in urinary infections[17,18]. The plant extract is rich in (polyphenols; flavonoids, and anthocyanins), and it has good antioxidant, and anti-inflammatory potential [19]. Several *in vitro* studies have affirmed the cytotoxic activity of anthocyanins, particularly against breast, lung, and GIT cancers [20]. Research based on the definition of phytonutrients and pharmacological actions of *Cordyline terminalis* is limited, which motivates authors to study *Cordyline terminalis's* different components. UPLC-ESI-QTOF-MS/MS has proven to be a prominent tool for natural compound identification due to its ionization as well as its high sensitivity and specificity.

So, for more information about the polyphenolic constituents, particularly "flavonoids," which could be useful in understanding their role and maximizing the plant*'s* usage for medicinal purposes, aqueous methanolic extract was used to visualize and study the phytochemicals of *Cordyline terminalis* leaves to support the de-replication of well-known metabolites. As far as we know, this is the first study to investigate the cytotoxic activity of *Cordyline terminalis* leaves fractions against liver, breast, and colon human carcinoma cell lines.

2. METHODS

2.1. Plant Material

Cordyline terminalis Kunth leaves were collected from Al-Zohriya Garden, Zamalek, Cairo, Egypt, during May and June (2018). Plant identification was done by Prof. Dr. Reem Samir Hamdy, Professor at the Botany and Microbiology Department of the Herbarium Faculty of Science at Cairo University. The voucher specimen (CT-18) was stored in the Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Egypt.

2.2. Extraction of Plant Leaves

One kg of dried plant leaves was extracted several times via (4 x 3L) with 70 % methanol under reflux. The extract was concentrated by a rotary evaporator (Büchi Co., Switzerland), and it was fractionated successively by petroleum ether, chloroform, ethyl acetate, and *n*-butanol according to polarity [21].

2.3. Experimental Method

UPLC-ESI-QTOF-MS/MS analysis was performed for profiling the secondary metabolites of *Cordyline terminalis* aqueous methanolic extract. Dissolve 100 mg of extract in 1 ml of mobile phase 1 (buffer HCOONH4 5 mM pH-8 in 1% methanol), vortexed at ordinary temperature, sonicated (10 minutes), centrifuged (5 minutes, 10,000 rpm), then 10 µl of solution (50/1000 µl) diluted with reconstitution solvent (1000 µl). At the end, the injected concentration was 2µg/2µl. The following multi-step gradient was used for mobile phase 1, with an increasing gradient of 10-90% for mobile phase 2 (100 acetonitrile). The rate of flow was ≥ 0.3 ml/min for 30 min. Pre-column filtration at disks in line 0.6 µm x 4 mm, X column HSS T3 2.6 µm, 2.2 x 150 mm (Waters co., Milford, USA), for separation, a column temperature of 40°C was used. Peak-View software.2.1 (SCIEX/The proteomics and metabolomics research unit at 57357 Hospital, Cairo, Egypt) for the LC-QTOF management; 10 µl was the injected volume of the LC system for both the test and mobile phases as a blank.

2.4. Investigation of the Cytotoxic Effect

The fractions were investigated for their cytotoxic activity by SRB assay against 3 different carcinoma cell lines: liver cells (HepG)-2, colon cells (HCT)-116, and finally breast cells (MCF)-7, according to the typical procedure described [22].

2.5. Insect rearing

Mosquito colony rearing and maintenance:

The research facility strain of *C. pipiens* was raised and kept up persistently for metagenesis at the Research and Training Center for Vectors of Diseases Insectary (RTC), Science College, Ain Shams University, utilizing the standard strategies portrayed by Kasapand Demirhan [23], to be maintained under conditions of 27±2 °C and RH 75±5% and photoperiod 12:12 light at dark [6]. The recently bred larvae were bolstered on tetramine. The collected pupae were turned into raised screened wooden bags (25×25×25 cm). Feeding adults on a 10% sucrose solution per day.

Larvicidal bioassay: *C. pipiens* larvae 3rd instar was given three different concentrations of *Cordyline terminalis* leaves aqueous methanol extract based on the standard protocol [24], with some modifications. *Cordyline terminalis* extract at different concentrations (50, 100, and 200 ppm) diluted with dist. water. Dist. water was the control. Each treatment and control contained 20 larvae, and we replicated the experiment three times. Detecting the lethal concentrations 24-h and 48-h after treatment.

3. RESULTS

3.1. UPLC-ESI-QTOF-MS/MS of Polyphenolics

Cordyline terminalis Kunth leaves aqueous methanolic extract was subjected to UPLC-QTOF-

ESI-MS/MS at both ion modes. Comparing R_t, and MS/MS fragments with reported data and an online database were used to detect the compound peaks, resulting in the identification of 44 and 32 compounds in negative and positive ion modes, respectively. These identified compounds were characterized as **flavonoids**: (luteolin-*C*-hexoside (21), vitexin-*O*-deoxyhexoside (23), luteolin (30), vitexin (38), daidzein-*C*-hexoside (47), ferulic acid (50), apigenin (54), diosmetin (56), acacetin (59), and kaempferol (64). Besides, flavonoids that were recognized at positive or negative ion modes (i.e., kaempferol-*O*-hexouronide (6), biacalein-*O*-hexouronide (15), luteolin-di-*O*-hexoside (25), kaempferol-*O*-neohesperidoside (32), **anthocyanins;** pelargonidin-*O*-dihexoside (29), delphinidin-*O*-rhamnosyl hexoside (34), cyanidine-*O*-hexoside (40), and peonidin-coumaroyl-hexoside (51), **isoflavones;** daidzein (20), besides **aurones, coumarines, stilbenes, terpenes, phenolic acids, organic acids, steroidal saponin**, and **saccharides**. **Figures 1& 2 / Table 1.**

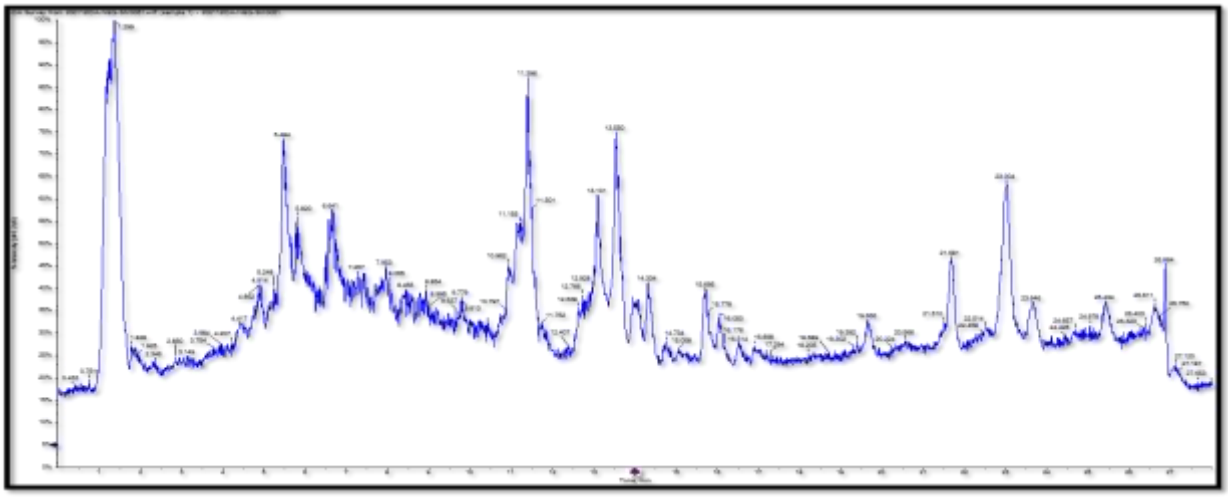

Figure 1. Negative mode UPLC-ESI-QTOF-MS/MS total ion chromatogram (TIC) of *C. terminalis* Kunth methanol leaves extract.

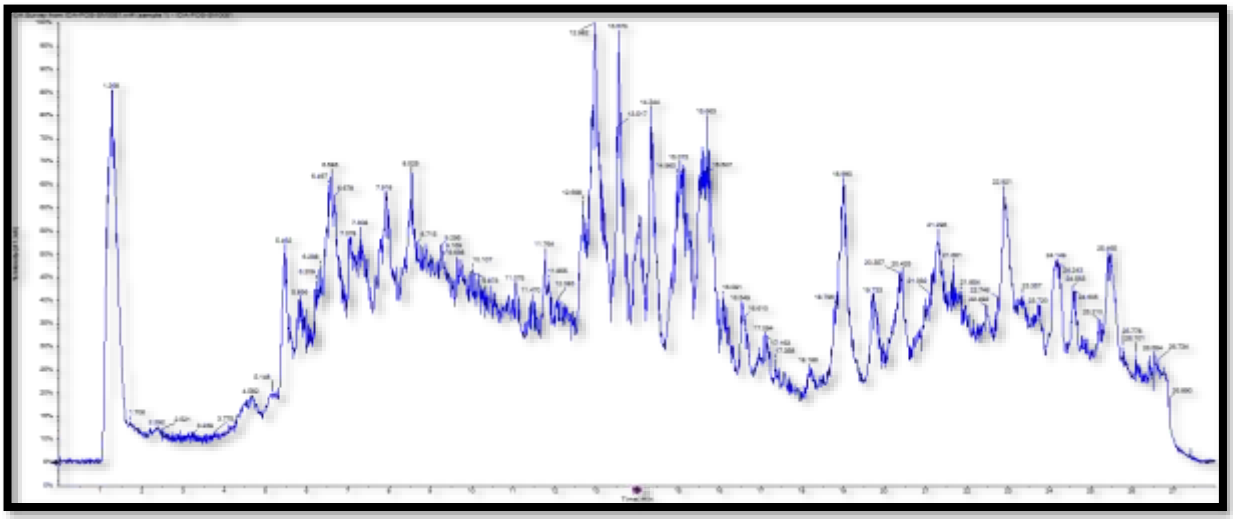

Figure 2. Positive mode UPLC-ESI-QTOF-MS/MS total ion chromatogram (TIC) of *C. terminalis* Kunth methanol leaves extract.

3.2. *In-Vitro* Cytotoxic Activity of Plant Leaves Extracts:

C. terminalis aqueous methanolic extract exhibited the most potent cytotoxic activity against HepG-2, HCT-116, and MCF-7 with IC_{50} values of 9.02, 10.98, and 11.13 µg/mL, followed by the petroleum ether fraction (IC_{50} = 9.28, 11.4, and 11.85 µg/mL), then butanol extract, which scored IC_{50} = 12.8, 13.9, and 14.1 µg/mL, and ethyl acetate extract had IC_{50} = 50.2, 25.6, and 22.6 µg/mL. Finally, chloroform fraction exhibited cytotoxic activity with IC_{50} of 113.6, 188.9, and 201.5 µg/mL, which was the highest IC_{50} value. The IC_{50} of the doxorubicin

reference standard was 0.36, 0.49, and 0.35 µg/mL **Table 2.**

3.3. *Larvicidal-assay*

The insecticidal activity of *Cordyline terminalis* Kunth leaves extract was determined against the third *C. pipiens* larvae instar at 24 and 48 hours after treatment, and the results are presented in Table 3. Larvae mortality increases with concentrations and exposure time. The results indicate that *Cordyline terminalis* leaves extract affected larval mortality rates with LC_{50} values of 268.96 and 248.43 ppm for 24-h and 48-h after application, respectively. At 24 h post-treatment, the toxicity index was 97.12, while at 48 h post-treatment, the toxicity index was 81.41, indicating that the toxicity index of leaves extract decreases with time.

Table 1. Tentatively identified compounds by negative and positive modes UPLC-ESI-QTOF-MS/MS in *C. terminalis* Kunth aqueous methanolic extract

NO.	RT (min)	Mol. Wt.	$[M-H]^-$	$[M+H]^+$	MS^n ions (*m/z*)	Identified compound
1	1.082	262.1	260.8698	-	215, 118	**Sorbitol-6-phosphate**
2	1.121	118.08	117.0294	-	99, 73	**Succinic acid**
3	1.134	134.08	133.0119	-	115, 71, 59	**Malic acid**
4	1.134	148.1	147.0316	-	129, 115	**Citramalic acid**
5	1.134	162.1	161.0447	-	143, 99	**Meglutol (3-hydroxy-3-methylglutaric acid)**
6	1.169	462.4	461.0567	-	461, 285, 153	**Kaempferol-*O*-hexouronide**
7	1.212	90.07	89.0228	-	------	**Lactic acid**
8	1.212	342.3	341.1066	-	305	**Trehalose**
9	1.216	148.07	149.0450	-	131	**Tartaric acid**
10	1.216	192.1	191.0567	-	------	**Isocitric acid**
11	1.229	210.1	209.0664	-	129, 101	**Mucate**
12	1.242	176.1	175.06 11	-	157,131,113	**Isopropylmalic acid**
13	1.307	146.1	145.0673	-	129	**Citramalate**
14	1.319	138.1	137.0328	-	78	**Urocanic acid**
15	1.320	446.4	445.1623	-	269	**Baicalein-*O*-hexouronide**
16	1.339	164.1	163.0695	-	119	**3-(4-hydroxyphenyl prop-2-enoic acid)**
17	1.359	182.1	181.0740	-	114	**Galactitol**
18	1.892	131.1	131.0719	-	129, 85	**2-Hydroxy-4-methylpentanoate**
19	2.285	152.1	151.0412	-	107	**Mandelic acid**
20	4.633	254.2	-	255.1210	127	**Daidzein**
21	4.978	448.3	447.1470	-	429, 357, 327	**Luteolin-*C*-hexoside**
22	5.121	434.3	433.1526	-	301	**Quercetin-*O*-pentoside**
23	5.376	578.5	577.1548	-	431, 149	**vitexin-*O*-deoxyhexoside**
24	5.592	425.4	424.1745	-	287	**Sinalbin**
25	5.863	610.5	609.3162	-	447, 285	**Luteolin-di-*O*-hexoside**
26	5.896	406.1	405.1789	-	-------	**E-3,4,5'-trihydroxy-3'-glucopyranosylstilbene**
27	5.913	464.3	-	465.1742	303	**Quercetin-3-*O*-glucoside**
28	6.171	840.5	839.3668	-	705, 561	**Dihydroxy spirost-ene dipentoside deoxyhexoside**
29	6.208	595.2	-	595.1649 $[M]^+$	433, 271	**Pelargonidin-*O*-dihexoside**
30	6.232	286.2	285.1659	-	153	**Luteolin**
31	6.284	184.17	-	183.0878	165, 151, 109, 95, 77	**Syringaldehyde**
32	6.495	594.5	-	595.3346	449,433,287,129	**Kaempferol-*O*-neohesperidoside**
33	6.521	302.2	-	303.0529	153	**Quercetin**
34	6.538	611.5	-	611.1622 $[M]^+$	465, 303	**Delphinidin-*O*-rhamnosyl hexoside**
35	6.689	180.1	179.0577	-	161, 135	**Caffeic acid**
36	6.797	180.2	-	181.0036	-------	***O*-Phenanthroline**

Table 1. cont.

NO.	RT (min)	Mol. Wt.	$[M-H]^-$	$[M+H]^+$	MS^n ions (*m/z*)	Identified compound
37	6.829	148.1	-	149.0316	131,105,77	**Trans-Cinnamate**
38	6.851	432.3	-	433.0963	415,397,343,337,313, 283,271,153	**Apigenin-8-*C*-glucoside (Vitexin)**
39	6.880	139.1	138.0230	-	-------	**P-nitrophenol**
40	7.130	449.3	-	449.1836 $[M]^+$	449, 287	**Cyanidin-*O*-hexoside**
41	7.258	624.5	-	625.1699	479, 317	**Isorhamnetin-*O*-rutinoside**
42	7.312	288.2	-	289.1939	103	**3',4',5,7-Tetrahydroxyflavanone**
43	7.345	272.2	271.1500	-	253, 151	**Naringenin**
44	7.440	448.3	-	449.1836	287	**Maritimetin-*O*-hexoside**
45	7.662	432.38	-	433.1492	433, 271	**Apigenin-*O*- hexoside**
46	7.715	228.2	-	229.1552	91	**Unknown**
47	7.837	416.4	-	417.1141	417, 255, 137	**Daidzein-*C*- hexoside**
48	7.881	408.5	407.17102	-	371	**Cholic acid**
49	7.995	145.1	-	146.0956	118	**Unknown**
50	8.345	194.1	-	195.0981	177, 165, 151, 91	**Ferulic acid**
51	8.357	769.7	769.4059	-	607,177	**peonidin coumaroyl hexoside**
52	8.872	182.1	181.0511	-	166, 151	**Syringaldehyde**
53	9.917	742.4	-	743.4452	597, 581, 435	**Fruticoside J**
54	10.091	270.2	269.0806	-	269, 253, 117	**Apigenin**
55	10.306	178.1	177.0547	-	145, 117	**Daphnetin**
56	10.570	300.2	299.0922	-	284, 237, 149	**5,7, 3'-trihydroxy-4'-methoxflavone (Diosmetin)**
57	10.928	612.5	611.1956	-	-	**Neohesperidin dihydrochalcone**
58	11.566	194.1	-	195.1337	81	**Unknown**
59	11.622	284.2	-	285.0750	270, 242, 153	**Acacetin**
60	12.557	756.6	-	757.4416	595, 433	**Trihydroxy furostene dihexoside**
61	12.673	360.3	359.1139	-	315, 192, 179	**Rosmarinic acid**
62	13.625	226.3	-	227.1591	83, 67	**Carnosine**
63	13.648	318.2	-	319.1601	145	**Myricetin**
64	13.779	286.2	285.0755	-	165, 137, 119	**Kaempferol**
65	13.922	376.3	-	377.1955	-	**Riboflavin**
66	14.082	386.3	-	387.1830	267, 147, 121	**1-*O*-*β*-D-glucopyanosyl Sinapate**
67	14.694	344.3	343.1235	-	-	**Maltitol**
68	14.707	822.9	822.4208	-	775, 685, 611, 577, 454, 397, 359, 197, 112	**Unknown**
69	15.593	722.1	721.4136	-	575, 429	**Fruticoside M**
70	16.615	342.3	-	343.3026	325, 307	**Trehalose**
71	16.655	340	339.1994	-	177	**Esculin**
72	18.793	626.5	624.921	-	463, 301	**Quercetin *O*-dihexopyranoside**
73	19.694	316.2	-	317.1149	-	**3,3',4',5-Tetrahydroxy-7-methoxyflavone**
74	22.620	178.1	-	179.0697	161, 133, 107, 91, 77	**Methoxycinnamic acid**
75	26.902	109.1	-	110.0616	55	**Hypotaurine**
76	26.938	152.1	-	153.1429	109	**4-hydroxy-3-methoxy benzaldehyde**

***** RT: retention time; Mol. Wt.: molecular weight; MS^n ions: ms/ms spectrometry ions

Table 2. *In vitro* cytotoxic activity of the plant extracts on the tested human cell lines HEPG-2, HCT-116 and MCF-7.

	IC_{50} µg/mL		
Sample	**HepG-2**	**HCT-116**	**MCF-7**
Aqueous methanolic extract	9.02 ± 1.05	10.98 ± 1.03	11.13 ± 0.8
Pet. ether fraction	9.28 ± 1.34	11.4 ± 1.1	11.85 ± 0.9
Chloroform fraction	113.6 ± 4.8	188.9 ± 6.5	201.5 ± 7.9
Ethyl acetate fraction	50.2 ± 1.6	25.6 ± 2.4	22.6 ± 2.8
Butanol fraction	12.83 ± 5.1	13.98 ± 6.9	14.1 ± 8.1
Doxorubicin	0.36 ± 0.04	0.49 ± 0.07	0.35 ± 0.03

Values are presented as mean ± SD of three tests.

Table 3. Larvicidal activity of *C. terminalis* leaves extract on 3rd larval instar of *C. pipiens* at 24 and 48 h post-treatment.

Extract (ppm)	**Leaves extract**	
	24 h post-treatment	**48 h post-treatment**
LC_{50} (*F.l. at 95%)	268.96 (82.34-295.24)	248.43 (219.85-278.76)
LC_{90} (*F.l. at 95%)	491.72 (83.05-2947.54)	453.58 (78.54-2831.83)
Slope ± SE	2.214±0.256	2.22±0.23
Probability (P)	0.16	0.11
Toxicity index	97.12	81.41
Relative potency	1.14	1.216

*(F.l.) Fiducially Limits

*Slope of the concentration-inhibition regression line ± standard error.

4. DISCUSSION

Profiling of Polyphenolic Compounds via UPLC-ESI-QTOF-MS/MS

The annotation of *Cordyline terminalis* Kunth secondary metabolites using UPLC-ESI-QTOF-MS/MS is the primary focus of this study. The comprehensive analysis is carried out in both positive and negative ion modes to provide a detailed tool for identifying its metabolites (see Figures 1 and 2; Table1).

Flavonoids:

A total of thirty flavonoid compounds, including the *C*-glycosyl type, were tentatively identified. The study aims to understand their fragmentation pathways, particularly those characterized by the ring-cleavage of sugar blocks at $[M-90/-120]^{-/+}$ and H_2O loss at $[M-18]^{-/+}$. For example, Compound 21 displayed characteristic ion peaks at R_t 4.978, and exhibited an $[M-H]^-$-ion at *m/z* 447.1470. The MS/MS spectrum showed peaks at *m/z* 357 $[M-H-90]^-$, *m/z* 327 $[M-H-120]^-$. That supported the presence of *C*-linked hexose as a sugar and luteolin as an aglycone, and was annotated as luteolin-*C*-hexoside [25]. Similarly, Comp. 38 was detected at R_t 6.851, revealing the $[M+H]^+$ ion at *m/z* 433.0963. In the MS/MS spectrum peaks were observed at *m/z* 343 $[M+H-90]^+$, at *m/z* 313 $[M+H-120]^+$. Additionally, other fragment ions were found at *m/z* 397, 337, 283, 271, and 153. These results confirm the cross-ring cleavage of hexose moiety in the *C*-link type, identifying it as apigenin 8-*C*-glucoside (Vitexin)[26].

The *O*-glycosyl type, in contrast to the *C*-glycosyl type, illustrates a distinctive fragmentation pattern resulting from the cleavage of specific sugar moieties. These include deoxyhexose or rhamnose side (-146 amu), hexose moiety (-162 amu), pentose or xylose moiety (-132 amu), hexouronide moiety (-176 amu), and neohesperidoside moiety (308 amu). This observed pattern of *O*-hexose attachment was noted in Comp. 25, 27, 29, 40, 44, 45, 51, and 72.

Upon analyzing comp. 25, it was identified at R_t 5.863 min with deprotonated $[M-H]^-$ ions at *m/z* 609.3162, and its MS/MS fragmentation ions at *m/z* 447 $[M-H-162]^-$, along with another fragment at *m/z* 285 due to the loss of two hexose molecules (324 amu). This compound was tentatively recognized as luteolin-di-*O*-hexoside [27]. Comp. 27, detected at R_t 5.913 min, showing its protonated ions $[M+H]^+$ at *m/z* 465.1742 and a fragment ion at *m/z* 303 resulting from the loss of a glucose moiety $[M+H-162]^+$, and was recognized as quercetin-3-*O*-glucoside, a compound previously isolated from plant [28, 29]. Apigenin-*O*-hexoside, identified at R_t 7.662 min,

displayed a protonated ion at *m/z* 433.1492 and a fragment ion at *m/z* 271 due to the cleavage of a hexose molecule [30]. Peak 22, identified as quercetin-*O*-pentoside, displayed the main ion at *m/z* 433.1526 and the fragment ion at *m/z* 301 $[M-H-132]^-$ [19].

Vitexin-*O*-deoxyhexoside was identified at R_t 5.376 min and produced deprotonated ions at *m/z* 577.1548 and its fragment ion at *m/z* 431 resulted from the cleavage of the rhamnosyl molecule (146 amu), along with a fragment at 149 [31]. The spectra for comp. 41 showed a molecular ion peak at *m/z* 625.1699 $[M+H]^+$ and a fragment peak at *m/z* 479 $[M+H-146]^+$, as a result of rhamnosyl moiety cleavage followed by hexoside loss, along with a peak at *m/z* 317 $[M+H-308]^+$, in agreement with the suggested identification as isorhamnetin-*O*-rutinoside [31].

Kaempferol-*O*-neohesperidoside exhibited its ions peak at *m/z* 595.3346, along with 449 $[M+H-146]^+$ corresponding to rhamnose loss, followed by hexose loss at *m/z* 287 $[M+H-146-162]^+$ due to the neohesperidoside moiety loss [32]. Kaempferol-*O*-hexouronide, identified at R_t 1.169 min, produced a deprotonated ion at *m/z* 461.0567 and a fragment ion at *m/z* 285 $[M-H-176]^-$ from the cleavage of hexouronide molecule, along with another fragment ion at *m/z* 153 due to RDA cleavage mechanism [29]. Baicalein-*O*-hexouronide, identified at R_t 1.320 min, yielded a deprotonated ion at *m/z* 445.1623 and its fragment ion at *m/z* 269, resulting from the cleavage of hexouronide molecule (-176 amu) [33].

Flavanones identified in our study as aglycones concerning 3', 4', 5, 7-Tetrahydroxyflavanone (eriodictyol) gave protonated at R_t 7.312 min at *m/z* 289.1939, characteristic for its molecular weight [34, 35]. Naringenin was identified in the negative mode at R_t 7.345 min; the precursor ion was at *m/z* 271.1500 $[M-H]^-$, which is distinctive for naringenin; and MS^2 fragment ions at *m/z* 253 $[M-H-18]^-$, characteristic for water loss; at *m/z* 151, the RDA cleavage mechanism for hydroxylation was explained [36, 37].

Anthocyanins: 4 compounds were identified. Regarding comp. 29, it was detected at 6.208 min, exhibiting protonated molecule $[M]^+$ at m/z 595.1649, fragmentation pattern at m/z 433 and 271 for loss of hexose moiety in its MS^2 spectra $[M-162]^+$ and $[M-162\times2]^+$ that was tentatively identified as Pelargonidin-*O*-dihexoside [28, 29].

Delphinidin-*O*-rhaminosyl hexoside was identified at R_t 6.538 min and gave protonated ion $[M]^+$ where the main peak appears at *m/z* 611.1622 and the product peak at *m/z* 465 $[M-146]^+$ characteristic for rhamnose cleavage, fragment ion at *m/z* 303 resulting from loss of rutinoside molecule $[M-308]^+$ [38]. Concerning comp. 40, which was detected at R_t 7.130 min and exhibits a protonated molecule $[M]^+$ at *m/z* 449.1836 and an MS^2 ion at *m/z* 287 by loss of hexose moiety, it was recognized as Cyanidin-*O*-hexoside[39]. Peonidin coumaroyl hexoside yielded MS/MS fragmentation ions that appeared at the negative mode ion at R_t 8.357 min and showed ion mass at *m/z* 769.4059 as the main peak and 607 as the fragment peak [39].
Isoflavones: two peaks were recognized as diadzein that appeared at R_t 4.633 min, which exhibited its base peak at *m/z* 255.1210 for protonated ion [40]. Diadzein-*C*-hexoside (puerarin) appeared at R_t 7.837 min, gave a protonated ion peak at m/z 417.1141, beside fragments at *m/z* 255, 137 by loss of hexose moiety (-162 amu) [41].

Steroidal saponins: these metabolites are the most characteristic in the *cordyline* genus [42], this class has been reported to be isolated from the genus [43, 44]. Four saponins were observed in the spectra of the plant. Regarding comp. 28, it gave deprotonation at R_t 6.171 min, *m/z* 839.3668, and MS^2 at *m/z* 705 $[M-H-132]^-$ and *m/z* 561 $[M-H-132-146]^-$. It was identified as dihydroxyspirost-ene dipentoside deoxyhexoside, which was isolated from *Cordyline stricta* [44].

Comp. 53 yielded protonated mass at R_t 9.917 min, main mass at *m/z* 743.4452, MS/MS at m/z 597 by loss of deoxy-hexoside moiety (-146 amu), m/z 581 by loss of hexoside moiety (162 amu), and at m/z 435 $[M+H-162-146]^-$. This fragmentation data is similar to the fragmentation mass ions of fruticoside J, which is a tetrahydroxy cholest-ene deoxy-hexoside. It has been isolated from the plant leaves [42].

Moreover, compound 69 exhibited deprotonation at R_t 15.593 min, giving the main fragment ion at *m/z* 721.4136, MS/MS at *m/z* 575 $[M-H-146]^-$, and at *m/z* 429 $[M-H-2\times146]^-$ by loss of two deoxyhexose molecules, this fragmentation is characteristic for dihydroxy spiro-stanene di-deoxyhexoside; it was recognized as spirostan-25(27)-ene-1β,3α-diol-1-*O*-α-L-rhamnopyranosyl-1 To 2-α-L-rhamnopyanoside (fruticoside-M); it has also been isolated already from the plant [43].

Phenolic acids: seven acids were detected at R_t 1.339, 6.689, 6.829, 8.345, 12.673, 14.082, and 22.620 min, yielding main mass ions at *m/z* 163.069 $[M-H]^-$, *m/z* 179.0577 $[M-H]^-$, *m/z* 149.0316 $[M+H]^+$, *m/z* 193.1715 $[M-H]^-$, *m/z* 359.1139 $[M-H]^-$, *m/z* 387.1830 $[M+H]^+$, and at *m/z* 179.0697 $[M+H]^+$ that are recognized as 3-(4-hydroxyphenyl prop-2-enoic acid), caffeic acid, trans-cinnamate, ferulic acid, rosmarinic acid, 1-*O*-*β*-*D*-glucopyanosyl sinapate, and methoxy-cinnamic acid, respectively.

Evaluation of Cytotoxic Activities:

The plant extract was identified to contain anthocyanin compounds, which are known for their cytotoxic effects, especially against the gastrointestinal tract and the breast cancer [20]. Furthermore, steroidal saponins have been evaluated as sources of cytotoxic compounds [45]. Aqueous methanolic extract and its successive fractions were investigated for their cytotoxic activity against three different carcinomas: MCF-7, HepG-2, and HCT-116. The values of IC_{50} are illustrated in **Table 2**. The results indicated that aqueous methanolic extract provided resistance against tumors of three different lines. It showed the highest cytotoxic activity, which may be due to the biological activity of polyphenolic and different flavonoid classes indicated at the plant, followed by petroleum ether fraction. However, the chloroform fraction exhibited the least cytotoxic activity.

Evaluation of Larvicidal Activity:

The *cordyline* plants are characterized by the presence of steroidal saponins, renowned for their insecticidal activity [42, 46, 47]. The larvicidal activity of *Cordyline terminalis* Kunth aqueous methanolic extract was evaluated at 24 and 48 hours after application against 3rd instar larvae of *C. pipiens* (**Table 3)**. Significantly, larval mortality increased with increasing extract concentration and exposure time. The toxicity index of plant leaves extract decreases with time. The slope values are low, indicating the homogeneity of the tested population. Phytochemicals such as phenolic acid, flavonoids, and saponins are known for their mosquito-repellent and insecticidal properties. All compounds are reported as toxic to insects and have insecticidal activities. It has already been reported that phenolic compounds can be potentially used for the control of insect pests on various crops [48].

5. CONCLUSIONS

UPLC-MS was employed to scrutinize several secondary metabolites in *C. terminalis*. The *in vitro* cytotoxic activity of *C. terminalis* aqueous methanol extract and its different fractions (chloroform, ethyl acetate, and *n*-butanol) demonstrated the significant cytotoxic inhibitory effect of aqueous methanolic extract against liver, colon, and breast carcinoma cell lines. Moreover, the insecticidal activity of the plant extract was indicated. Our results may aid in assessing the various benefits of *C. terminalis* leaves as anticancer and larvicidal agents. Nevertheless, further researches are required to identify its biological activity in *in vivo*.

Supplementary Materials: Figures represent the MS-MS spectrometry of some tentative identified compounds in *C. terminalis* leaves.

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