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EMILIA PRAETERMISSA MILNE-REDHEAD (ASTERACEAE): PHYTOCHEMICAL PROFILING AND CYTOTOXIC POTENTIAL

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Abstract

Background: *Emilia praetermissa Milne-Redhead,* is a fast growing herb that is native to west and central Africa, the leaf is use in the treatment of ulcer, hernia, pain and tumor by traditional healers in these regions. It is explored in this study for its phytochemicals and cytotoxic activities.
Materials and Methods: The powdered leaf was screened for phytochemicals using standard methods, while profiling of the methanol extract was done by both Gas Chromatography-Mass Spectrometry (GCMS) and High Pressure Liquid Chromatography (HPLC). Cytotoxic potential was evaluated using *Raniceps raninus* (Tadpole-fish) at varying doses (20-320 µg/mL) of the extract (methanol) and fractions (ethylacetate and dichloromethane).
Results: Tannins, flavonoids, steroids, glycosides, alkaloids and saponins were revealed by the phytochemical screening. The GC-MS analysis identified thirty-three compounds, which include aziridine, benzaldehyde, benzofuran, carbazole, hydroquinone, isopropylidine, indole, piperidine, piperazine, pyrazole, pyridine, pyrrolidine and tetrazole derivatives. Twelve compounds were identified and quantified from the HPLC analysis; coumaric acid (10.2385 µg/ml), cresol (25.7350 ppm), ellagic acid (3.9547 µg/ml), ferulic acid (8.9514 µg/mL), isoflavone (22.7694 ppm), naringenin (14.1526 µg/mL), naringin (5.6179 µg/ml), pyrogallol (12.5850 µg/g), resveratrol (3.8639 ppm) and salicylic acid (17.4195 µg/mL) respectively. A dose of 160 µg/mL of the methanol extract significantly ($P<0.05$) resulted in (60.00 ± 5.78) % mortality rate within 90 minutes, and (100.00 ± 0.00) % mortality as the dose was double (320 µg/mL) after 120 minutes.
Conclusion: These thus indicate the presence of phytochemicals in *E. praetermissa* leaf with cytotoxic potential.

Keywords: Cytotoxic effect, *Emilia praetermissa*, Phytochemical profiling, *Raniceps raninus*, HPLC.

Abbreviation: GC: Gas Chromatography, MS: Mass Spectrometry, GC-MS : Gas Chromatography-Mass Spectrometry; HPLC : High Pressure Liquid Chromatography, RNA : Ribonucleic acid, DNA : Deoxyribonucleic acid, NIST : National Institute of Standard Technology, UV: Ultra violet, DMSO : Dimethylsulphoxide, ANOVA : Analysis of Variance, CNS : Central Nervous System, NO : Nitric oxide, 5HT1A : Serotonin 1A receptor, AMPK : Adenosine Monophosphate Protein Kinase, HIF-1α : Hypoxia-Inducible Factor 1-Alpha, LC50 : Lethal Concentration 50.

Introduction

Emilia praetermissa Milne-Redhead in the family Asteraceae, commonly and locally called yellow thistle and Odundun (Yoruba speaking people from Southwestern Nigeria). Native to west and central Africa, originally Nigeria, Cameroun, Garbon, Zaire, Togo, Ivory Coast and Sierra Leona, and naturalized in St Lucia, St Vincent, Taiwan and Martinique (Chung *et al.*, 2009; Graveson, 2016, POWO, 2020). Though erect annual fast growing herb that rapidly colonizes fallow or agricultural land as weeds, it is referred to as an invasive species in Taiwan and St Lucia. *Emilia praetermissa* Milne-Redhead is considered to be a tetraploid hybrid of *Emilia lisowskiana* and *Emilia sonchifolia* (Mapaya and Cron, 2016) and looks similar to other species like *Emilia sonchifolia*, *Emilia coccinea* and *Emilia*

fosbergii (Flora of China Editorial Committee, 2020). The leaf is use in Africa traditional medicines to treat ailments such as ulcer, sores, sinusitis, tumor, hernia, backache, pain, convulsion, enlarge spleen, vertigo, febrifuge and mixed with *Ipomoea eriocarpa* as an eye drop, while the root decoction are used to treat colic in babies and venereal disease. The leaves are either eaten fresh as salad or prepared as vegetables (Ruffo *et al*., 2002; Lebeau *et al*, 2017). Alkaloids, saponins, tannins, glycosides, flavonoids and steroids have been reported in the leaf of *E. praetermissa* Milne-Redhead (Ikezu, 2023).

The use of plants as medicines is gaining ground due to its benefits and its believed safety potential to its user (Sofowora *et al*., 2013). The benefits could be ascribed to the presences of phytochemicals (Leitzmann, 2016), that have been linked to many of its biological potentials (Kumar *et al*., 2023). These phytochemicals are grouped into different chemical classes. Earliest phytochemicals that have been used in the management of non-communicable disease like cancer include diterpenes-paclitaxel, vinca alkaloids (vinblastine, vinflunine, vincristine and vindesine) and flavone-flavopiridol. These are cytotoxic in nature through cell cycle specific, antimitotic and RNA transcription hampering effects. Their effect on cancerous cells could lead to apoptosis which is the aim of most chemotherapeutic drugs. Many plant species in the Asteraceae family, have reported cytotoxic potentials, but paucity exist in the cytotoxic study and profiling of *E. praetermissa* Milne-Redhead. Thus, this study aims to investigate the profiling and cytotoxic effects of *E. praetermissa* Milne-Redhead.

Materials and methods

Collection and identification of plant sample

Emilia praetermissa Milne-Redhead leaves were collected in September, 2023 from the new pharmacy building in the main campus of the University of Benin, with latitude: 6° 20’ 1.32” N, longitude: 5° 36’ 0.53” E. It was identified and authenticated by Dr Emmanuel Aigbokhan of the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin. Plant specimen was deposited in the Departmental herbarium, with herbarium number UBH-E407 assigned.

Preparation, extraction, concentration and fractionation of the plant sample

The leaves were carefully plucked from the whole plant, dried for two weeks under shade and pulverized using an electric milling machine into fine powder. The pulverized powder (200 g) was macerated in methanol (300 mL) for three days. The mixture was decanted, passed through filter paper (size 1) and concentrated in-vacuum at 50 °C using a rotary evaporator. The weight of the extract obtained was noted (10 g) and sample was kept in the refrigerator at 4°C when not in use.

Seven gram (7 g) of the methanol extract was solubilized in methanol (20 mL) and water (40 mL), this mixture was partitioned into n-hexane (5x60 mL). These were repeated for dichloromethane and ethylacetate fractions. The weights for each fraction were noted following their concentration in a rotary evaporator and were preserved in the refrigerator at 4°C until use.

Phytochemical screening of powdered leaf of *E. praetermissa*

The pulverized leaf sample was screened for alkaloids, flavonoids, glycosides, saponins, steroids, tannins and terpenoids using standard methods described by Sofowora (1993) and Trease and Evans (2009).

Gas Chromatography-Mass Spectrometric Analysis of the methanol leaf extract

Triple axis detector Gas Chromatography-Mass Spectrometry was used in the analysis of the methanol leaf extract of *E. praetermissa* Milne-Redhead. The model of the GC is Agilent USA 7890 system while the MS is an inert MSD. Auto injector syringe of 10 mL was outfitted, with Helium as the carrier gas and Agilent (19091-433HP-5Ms) capillary column activated with phenyl polymethyl siloxane was utilized in the separation. The specification of the capillary column was as follow; length (30 mm), internal diameter (0.2 µm) and thickness (250 µm). Ion source temperature was 250°C, interface temperature was 300°C, Pressure was maintained at 16.2 psia, split mode injection of 1 µL, split ratio of 1:50 and temperature at injection point was 280°C. Column temperature of 50°C was set for 2 mins; this was increased to 100°C at 20°C/min rate.

With rate left constant (20°C/min), the temperature was increased to 250°C and kept constant for 5 mins. Setup was adjusted by the manufacturer software solution of the mass spectrometry and the data were gotten in the process. Compounds were identified by mass spectra comparison of each compounds and standard from NIST library (Odion *et al*., 2024).

High Pressure Liquid Chromatographic analysis of the methanol leaf extract of *E. praetermissa.*

The components of the High Pressure Liquid Chromatography (HPLC) include a column oven, twofold binary pumps and an UV/Visible detector (Shimadzu: LC-10AD; CTO-10AS; SPD-20A). C-12 Normal phase column

measuring 200 mm in length, 4.8 mm in internal thickness and 5 µ in thickness was utilized during the assay. Acetic acid-acidified deionized water mix, with pH of 2.8 were part of the mobile phase A and acetonitrile was used as the other mobile phase B at a flow rate of 0.8 mL/min. 5 % of B was used to balance the column for 20 min. following each injection of sample. Wavelength of 280 nm, temperature of the column was set at 38ºC and injection volume of 20 µL. Peak areas and retention times compared with external standards calibration plot were used in the quantification and identification. The standards are ammodendrine, anthocyanidine, anthocyanin, aphylidine, catechin, cardiac glycoside, cyanogenic, dihydrocytisine, flavone, glycoside, kaempferol, naringenin, phytate, quercetin, ribalinide, sapogenin, spartein, steroids and tannin (Odion *et al.*, 2024).

Gradient elution:

SN	Time (min)	Solvent B %	Solvent A%
1	0-5	5-9	91-95
2	5-15	9	91
3	15-22	9-11	89-91
4	22-38	11-18	82-89
5	38-43	18-23	77-82
6	43-44	23-90	10-77
7	44-45	90-80	10-20
8	45-55	100	0

Cytotoxic evaluation of the methanol extract and fractions of *E. praetermissa* leaf using *Raniceps raninus* (Tadpole-fish)

Three to four (3-4) days old *Raniceps raninus* of similar length and size were utilized for this experiment. Five *Ranicep raninus* were used in each group (I, II, III, IV, V, VI) for this experiment, with each labelled group placed in a 30 mL beaker containing natural water and 19 mL of water from its habitat. This was subsequently made to mark by adding 1 ml of the methanol extract or n-hexane and dichloromethane fractions (1-16 mg/mL) dissolved in 5 % dimethylsulphoxide (DMSO). The sixth group was used as control which involves dissolving 5 % DMSO in distilled water (50 mL). This setup was done in triplicate and at ambient temperature for 24 h. Mortality were observed, expressed as the mean± standard error of mean (Mean±SEM), which was used as a measure of cytotoxicity (Gbolade *et al*., 2019).

Statistical Analysis

Cytotoxic test was done in triplicate and results expressed as mean ± standard error of mean. Analysis of the data was done by one way ANOVA, while multiple comparison was done using Dunnett Posthoc test and level of significance set at $P<0.05$. Data were plotted and analyzed using Graphpad Prism Software 5.01 version.

Results

The powdered leaf of *E. praetermissa* contains phytochemicals as shown in Table 1.

Table 1: Phytochemical screening of *Emilia praetermissa* powdered leaf

Phytochemicals	Inferences
Alkaloids	+
Glycosides	+
Tannins	+
Saponins	+
Steroids	+
Flavonoids	+
Terpenoids	-

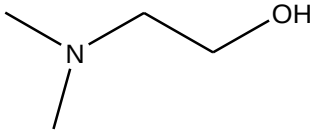
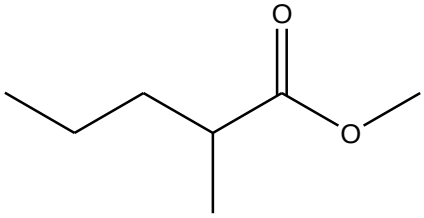
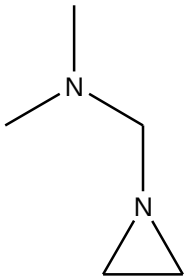
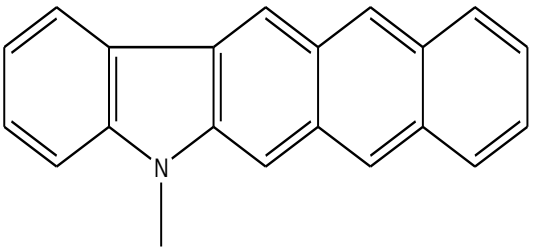
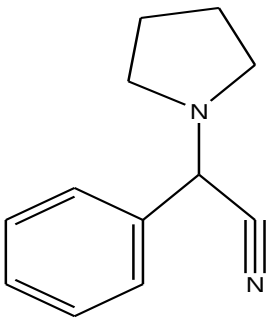
The GC-MS analysis of the methanol leaf extract of *E. praetermissa* Milne-Redhead yielded thirty-three compounds as shown in table 2. The compounds which include aziridine, benzaldehyde, benzofuran, carbazole, hydroquinone, isopropylidine, indole, piperidine, piperazine pyrazole, pyridine, pyrrolidine and tetrazole derivatives.

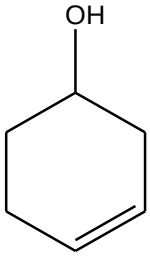
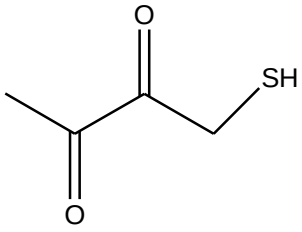
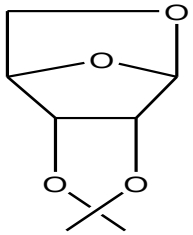
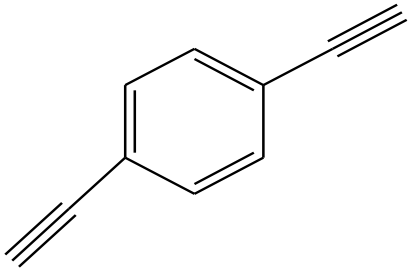
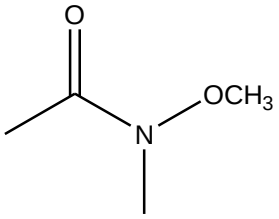
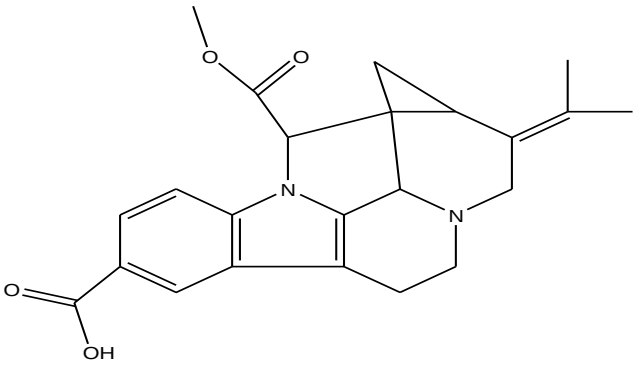
Table 2a: Compounds from GC-MS analysis of the methanol extract of *E. praetermissa*

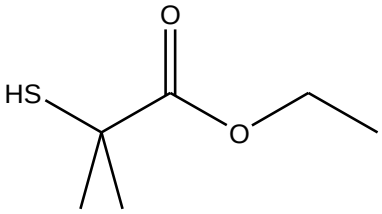
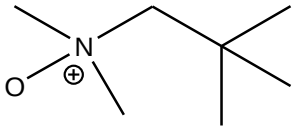
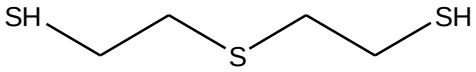
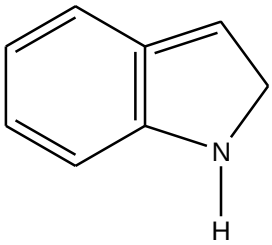
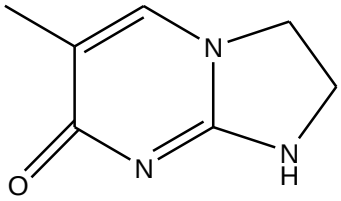
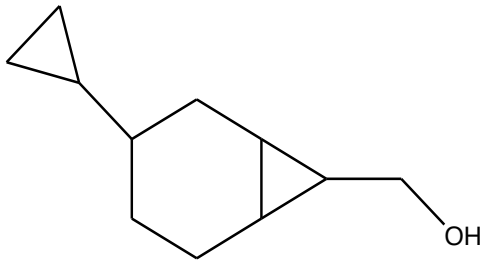
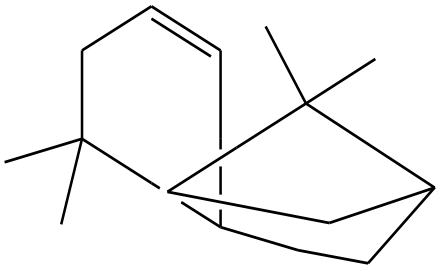
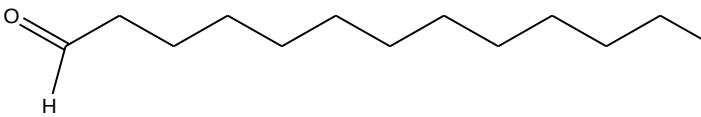
S/N	Compounds	Retention Time	Percentage Area	Molecular Formula	Molecular Weight
1	N,N-dimethylaminoethanol	2.510	0.78	$C_4H_{11}NO$	89.1362
2	Spiropentanoic acid methyl ester	3.130	0.07	$C_7H_{10}O_2$	126.1531
3	N-[Dimethylaminomethyl]aziridine	3.271	0.12	$C_5H_{12}N_2$	100.16
4	5H-Naphtho[2,3-c]carbazole,5-methyl	3.384	0.06	$C_{21}H_{15}N$	281.3
5	1-pyrrolidinylacetonitrile	3.637	0.18	$C_6H_{10}N_2$	110.16
6	3-cyclohexene-1-methanol	3.862	0.51	$C_7H_{12}O$	112.17
7	2,3-Dioxobutanethiol	4.060	1.43	$C_4H_6O_2S$	118.16
8	Ribofuranose,15-anhydro-2,3-O-isopropylidene-,d	4.313	0.78	$C_8H_{12}O_4$	172.18
9	Benzonitrile,4-ethenyl	4.989	3.44	C_9H_7N	129.1586
10	N-methoxy-N-methylacetamide	5.440	7.93	$C_4H_9NO_2$	103.12
11	1,16-cyclocorynan-17-oic acid, 19,20-didehydro-,methylester, (16S,19E)-	5.750	2.42	$C_{20}H_{24}N_2O_2$	324.42
12	Propanoic acid, 2-mercapto-,methylester	5.891	1.74	$C_4H_8O_2S$	120.170
13	Propylamine,N,N,2,2-Tetramethyl,N-oxide	6.032	2.34	$C_7H_{17}NO$	131.22
14	Ethanethiol,2,2'-thiobis	6.285	2.44	$C_4H_{10}S_3$	154.317
15	Indole	6.454	1.33	C_8H_7N	117.15
16	Imidazo(1,2-a)pyrimidine,6-methyl-5-oxo-,1,2,3,5-tetrahydro	6.679	4.09	$C_8H_{10}N_2O$	150.18
17	Bicyclo[4.1.0]heptane,-3-cyclopropyl,-7-hydroxymethyl,trans	6.820	1.29	$C_{11}H_{18}O$	169.28
18	8,9-dehydrocycloisolongifolene	7.074	4.50	$C_{15}H_{22}$	202.33
19	Tetradecanal	7.299	5.42	$C_{14}H_{28}O$	212.37
20	Hydroquinone	7.665	4.41	$C_6H_6O_2$	110.11
21	Hydroquinone, monoacetate	7.891	20.63	$C_8H_{10}O_4$	170.16
22	Bicyclo[3.1.1]heptane,2,6,6-trimethyl-(1α,2β,5α)	9.299	18.58	$C_{10}H_{18}$	138.25
23	Acetic acid 4-hydroxy-1-methyl-2-oxo-4-phenylpiperidin-3-ylester	10.285	9.31	$C_{15}H_{19}O_4N$	277.34
24	1,3,4-oxadiazol-2-amine,5-(1-phenyl-5-tetrazolyl)-	11.412	4.02	$C_9H_7N_7O$	229.20
25	6-cyanobenzofuran-1-oxide	13.722	0.41	$C_{16}H_{32}O$	240.42
26	2-aminocyclopentanemethanamide	14.652	0.61	$C_6H_{14}N_2$	114.19
27	1,2-dimethyl-2-pyrroline	14.990	0.18	$C_6H_{11}N$	97.16

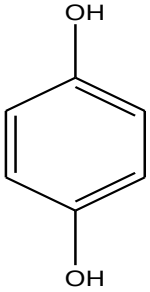
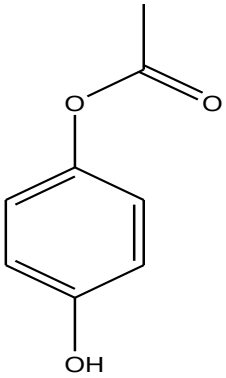
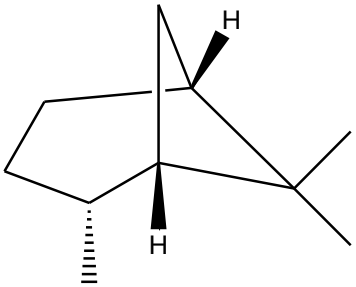
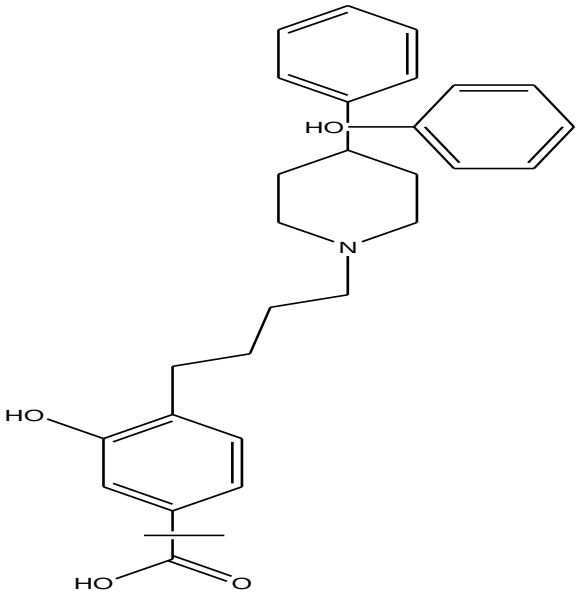
28	4-bromo benzaldehyde	15.553	0.08	C_7H_5OBr	185.02
29	1-butyl-3,4-dihydroxy-pyrrolidine-2,5-dione	15.722	0.03	$C_8H_{13}NO_4$	187.19
30	1H-pyrazole-4,5-dione,1-phenyl-4-(phenylhydrazone)	16.004	0.04	$C_{15}H_{12}N_4O$	264.28
31	4-Chloropyridine	16.145	0.02	C_5H_4ClN	113.54
32	Piperazin-1,4-diium,1,4-di(2-chloroethyl)-1,4-dimethyl-,dichloride	16.398	0.01	$C_{10}H_{22}Cl_4N_2$	312.10
33	N-methyl-1-adamantaneacetamide	16.877	0.02	$C_{13}H_{21}NO$	207.31

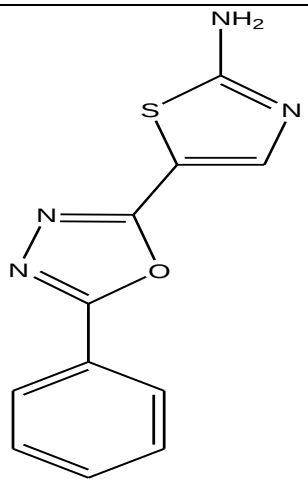
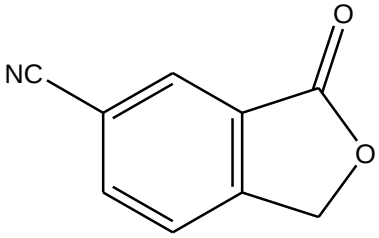
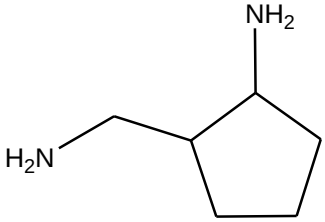
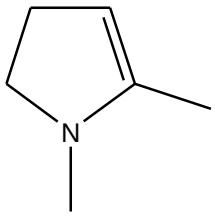
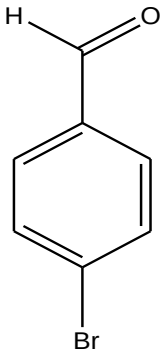
Table 2b: Structure of compounds identify by GC-MS analysis from *Emilia praetermissa* Milne-Redhead leaf extract

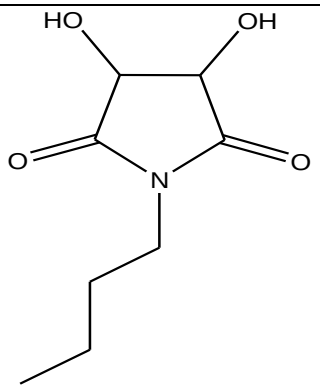
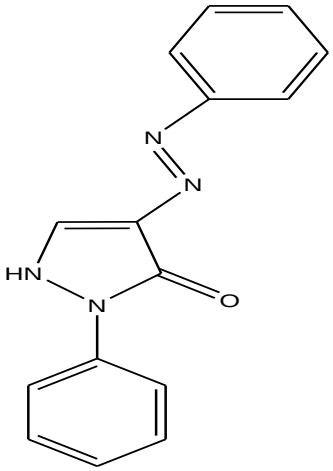
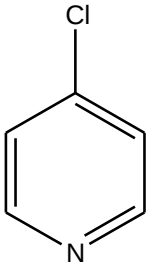
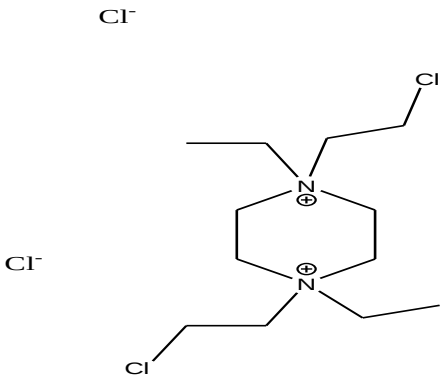
1	N,N-dimethylaminoethanol	
2	Spiropentanoic acid methyl ester	
3	N-[Dimethylaminomethyl]aziridine	
4	5H-Naphtho[2,3-c]carbazole,5-methyl	
5	1-pyrrolidinylacetonitrile	

6	3-cyclohexene-1-methanol	
7	2,3-Dioxobutanethiol	
8	Ribofuranose,15-anhydro-2,3-O-isopropylidene-,d	
9	Benzonitrile,4-ethenyl	
10	N-methoxy-N-methylacetamide	
11	1,16-cyclocorynan-17-oic acid, 19,20-didehydro-,methylester, (16S,19E)-	

12	Propanoic acid, 2-mercapto-,methylester	
13	Propylamine,N,N,2,2-Tetramethyl,N-oxide	
14	Ethanethiol,2,2’-thiobis	
15	Indole	
16	Imidazo(1,2-a)pyrimidine,6-methyl-5-oxo-,1,2,3,5-tetrahydro	
17	Bicyclo[4.1.0]heptane,-3-cyclopropyl,-7-hydroxymethyl,trans	
18	8,9-dehydrocycloisolongifolene	
19	Tetradecanal	

20	Hydroquinone	
21	Hydroquinone, monoacetate	
22	Bicyclo[3.1.1]heptane,2,6,6-trimethyl-(1α,2β,5α)	
23	Acetic acid 4-hydroxy-1-methyl-2-oxo-4-phenylpiperidin-3-ylester	

24	1,3,4-oxadiazol-2-amine,5-(1-phenyl-5-tetrazolyl)-	
25	6-cyanobenzofuran-1-oxide	
26	2-aminocyclopentanemethanamide	
27	1,2-dimethyl-2-pyrroline	
28	4-bromo benzaldehyde	

29	1-butyl-3,4-dihydroxy-pyrrolidine-2,5-dione	
30	1H-pyrazole-4,5-dione,1-phenyl-4-(phenylhydrazone)	
31	4-Chloropyridine	
32	Piperazin-1,4-diium,1,4-di(2-chloroethyl)-1,4-dimethyl-,dichloride	

The structures of these compounds are provided in supplementary file AJTCAM 1

Qualitative and quantitative analysis of the methanol extract of *E. praetermissa* Milne-Redhead leaf by HPLC showed twelve compounds as indicated in table 3. However, only ephedrine lack cytotoxic activity, while coumaric acid, cresol, ellagic acid, ferulic acid, isoflavone, naringenin, naringin, pyrogallol, resveratrol and salicylic acid showed cytotoxic potentials in selected cells.

Table 3: Compounds identified and quantified by HPLC analysis

S/N	Compounds	Retention Time	Concentration
1	Ephedrine	0.116	2.3565 µg/mL
2	Ribalinidine	3.686	2.7733 µg/mL
3	Cresol	7.893	25.7350 ppm
4	Ellagic acid	9.223	3.9547 µg/mL
5	Naringin	10.680	5.6179 µg/mL
6	Coumaric acid	15.126	10.2385 µg/mL
7	Isoflavone	20.356	22.7694 ppm
8	Ferulic acid	27.390	8.9514 µg/mL
9	Pyrogallol	28.253	12.5850 µg/g
10	Naringenin	37.656	14.1526 µg/mL
11	Salicyclic acid	42.360	17.4195 µg/mL
12	Resveratrol	43.540	3.8639 ppm

The methanol leaf extract of *E. praetermissa* Milne-Redhead had a dose-dependent cytotoxic effect. This can be seen from the 80 µg/mL dose, which showed (40.00 ± 3.62) % cytotoxicity within 1.5 h of exposure to the extract and obtained 100 % mortality in 24 h. The 160 µg/mL dose of methanol leaf extract of *E. praetermissa* Milne-Redhead resulted in a statistically significant ($P<0.05$) mortality rate of (60.00 ± 5.78) % within 1.5 h and (100.00 ± 0.00) % within 2.5 h in the tadpoles. The 320 µg/mL concentration exerted (56.67 ± 3.33) % mortality within 1 h and (100 ± 0.00) % death after 1.5 h, respectively. The tadpole-fish were not affected by the control (5 % DMSO) and 20 to 40 µg/mL dose of methanol extract of *E. praetermissa* Milne-Redhead.

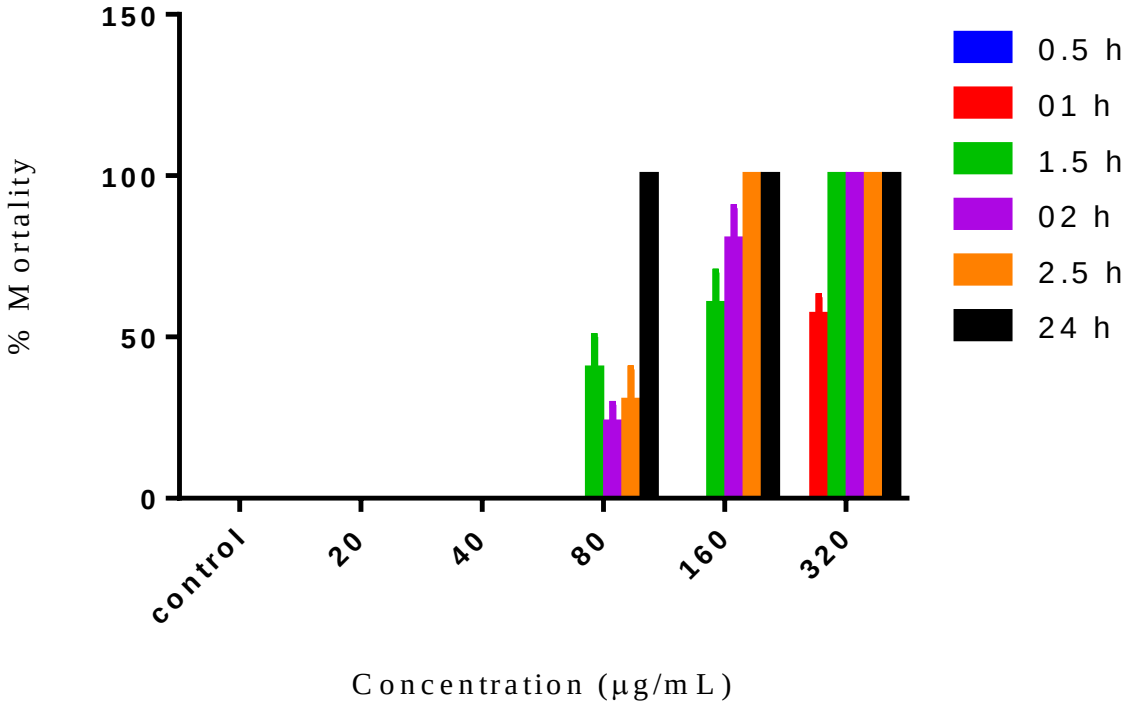


Figure 1: Cytotoxic effect of methanol leaf extract of *E. praetermissa* on *R. raninus*. Each bar represents mean ± SEM. Data is significant at $p<0.05$. n=10

The ethylacetate fraction of *E. praetermissa* Milne-Redhead had a dose-dependent cytotoxic effect. The 160 µg/mL concentration of ethylacetate fraction of *E. praetermissa* resulted in a statistically significant (P<0.05) mortality rate of (56.67 ± 3.33) % within 1 h and (100.00 ± 0.00) % within 1.5 h in the tadpoles. The 320 µg/mL concentration exerted (33.33 ± 3.33) % mortality after 0.5 hour and 100 % death after 1 h, respectively.

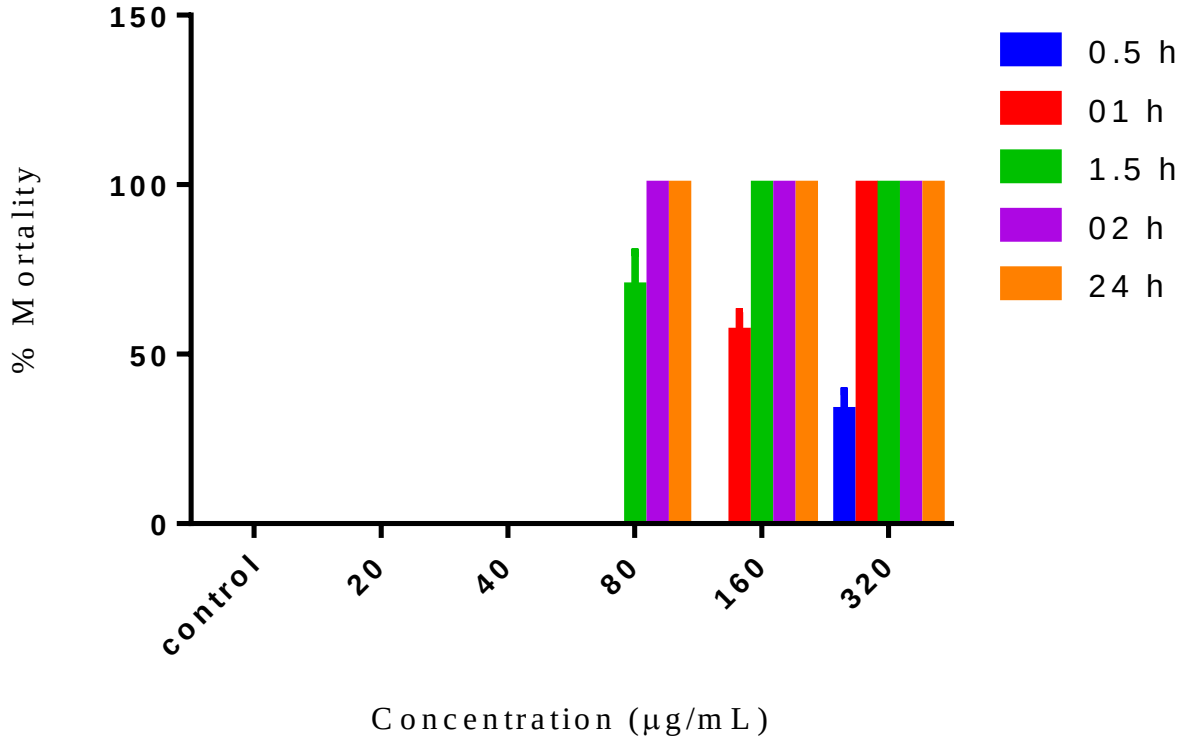


Figure 2: Cytotoxic effect of Ethylacetate fraction of *E. praetermissa* on *R. raninus*. Each bar represents mean ± SEM. Data is significant at p<0.05. n=10

The Dichloromethane fraction of *E. praetermissa* Milne-Redhead had a dose-dependent cytotoxic effect, according to the results. The 80 µg/mL concentration of the methanol leaf extract resulted in a statistically significant (P<0.05) mortality rate of (23.33 ± 6.67) % within 1.5 h and (100.00 ± 0.00) % within 2 h in the tadpoles. The 160 µg/mL concentration exerted (16.67 ± 3.33) % mortality after 0.5 h and 100 % death after 1.5 h, respectively.

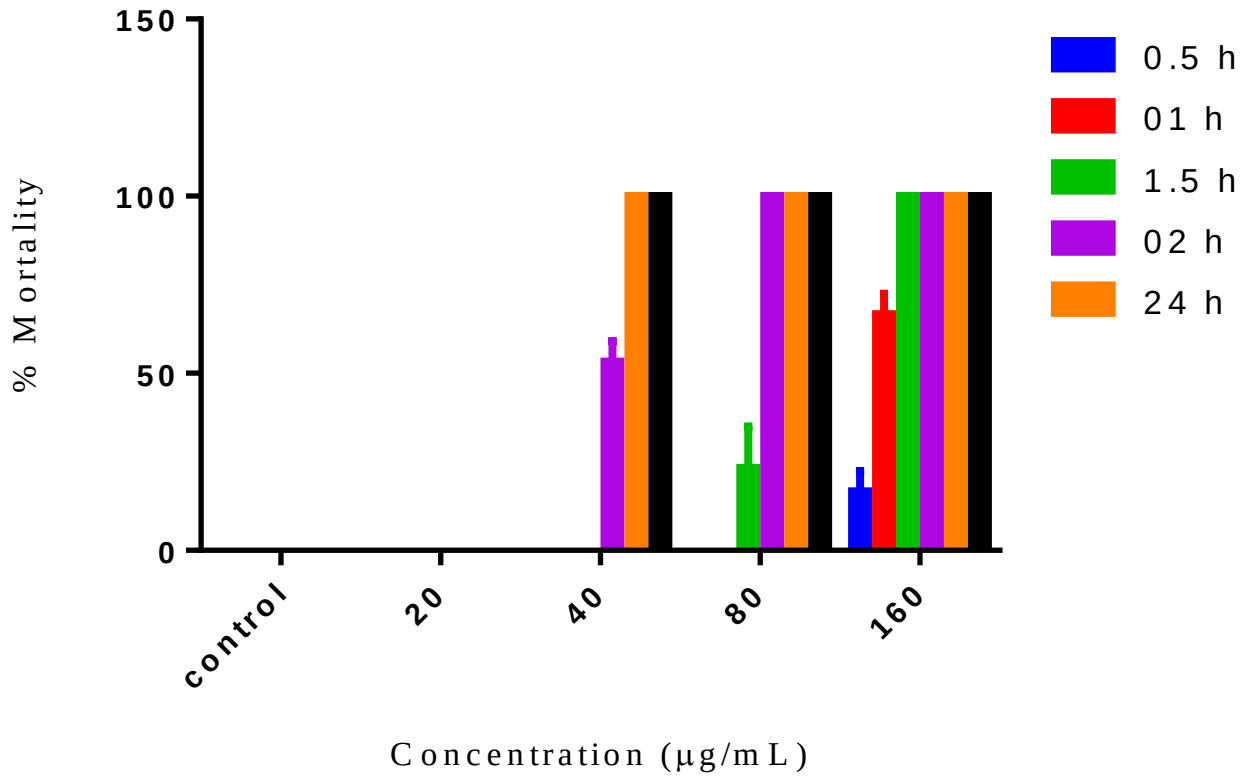


Figure 3: Cytotoxic effect of dichloromethane fraction of *E. praetermissa* on *R. raninus*. Each bar represents mean ± SEM. Data is significant at $p<0.05$. n=10

Discussion

Preliminary phytochemical screening of the leaf extract previously, revealed the presence of flavonoids, tannins and saponins (Lebeau *et al*., 2016). These results are in agreement with our test result, though not elaborate since it was only three of the tests that were conducted. However, in a more elaborate test carried out by smith (2021), the leaves of *E. praetermissa* Milne-Redhead showed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids and steroids. These results differ only in the presence of terpenoids; this could be due to difference in the geographical location. Presence of these phytochemicals in this plant is important, considering the protective effect they confer on the plant against predators. Also phytochemicals have various pharmacological activities, some include alkaloids-anticancer; flavonoids-antioxidant; tannins-anti-obesity; saponins-wound healing and steroids-antibacterial, thus making phytochemicals a reservoir of undiscovered drugs.

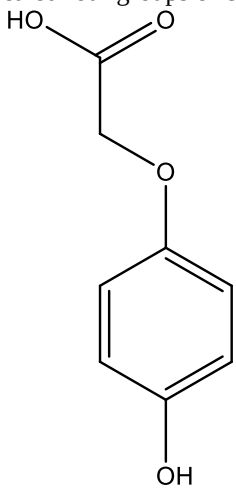
Profiling of plants part for its chemical constituents enable the understanding of the pharmacology or mechanism of action and development of new therapeutic potential for the plants. Extracts from *E. praetermissa* Milne-Redhead have been associated with different traditional and pharmacological uses. This study was able to identify thirty-three compounds from the leaves of *E. praetermissa* Milne-Redhead. The prominent compounds within the range of 3.00 % to 21.00 %, summing up to 76.91 %, include Hydroquinone, monoacetate (20.63 %); Bicyclo[3.1.1]heptane,2,6,6-trimethyl-(1α,2β,5α) (18.58 %); Acetic acid 4-hydroxy-1-methyl-2-oxo-4-phenylpiperidin-3-ylester (9.31 %); N-methoxy-N-methylacetamide (7.93 %); Tetradecanal (5.42 %); 8,9-dehydrocycloisolongifolene (4.50 %); Hydroquinone (4.41 %); Imidazo(1,2-a)pyrimidine,6-methyl-5-oxo-,1,2,3,5-tetrahydro (4.09 %); 1,3,4-oxadiazol-2-amine,5-(1-phenyl-5-tetrazolyl) (4.02 %) and Benzonitrile,4-ethenyl (3.44 %) respectively. However previous studies have presented compounds from the ethanol leaves of *E. sonchifolia*, the compounds identified include Dopexamine (Dopamine analogue-reduce exacerbation of heart failure), Propranolol (β-blocker, for management of hypertension), Dextroamphetamine (enantiomer of amphetamine-potent CNS stimulant), Pimozide (Neuroleptic drug), Sulfociprofloxacin 3β, 5β-(antibiotic), Penicillamine cysteine disulfide (antibiotic) and Dextromoramide (Analgesic) (Jeena *et al*., 2023). The compounds identified in this study include N,N-dimethylaminoethanol, a tertiary amine used as radical scavenger. Pyrrolidine-2,5-dione moiety from 1-butyl-3,4-dihydroxy-pyrrolidine-2,5-dione have been shown

to be potent 5HT1A receptor and serotonin transporter protein ligands (Wrobel *et al.*, 2013). 1H-pyrazole-4,5-dione,1-phenyl-4-(phenylhydrazone) with the pyrazole nucleus conferring anti-inflammatory, analgesic, anti-obesity, antipsychotic and antidepressant activities on this molecule (Karrouchi *et al*., 2018). Chloropyridine is an example of non-benzodiazepam with pharmacological profile that is similar to chlordiazepoxide and nitrazepam use as CNS drugs. Piperazin-1,4-diium,1,4-di(2-chloroethyl)-1,4-dimethyl-,dichloride analogues of 1-benzylpiperazine is use as stimulant (Gee and Schep, 2022). N-methyl-1-adamantaneacetamide analogue possess activity against Dangue mosquito larva (cidal and repellant properties) (Chellappandian *et al*., 2022). Some of these activities are undocumented pharmacological potentials of *E. praetermissa* Milne-Redhead.

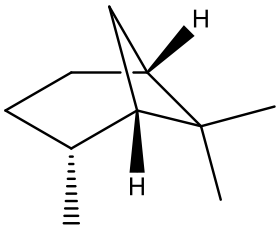
Hydroquinone acetate and Bicyclo[3.1.1]heptane,2,6,6-trimethyl-(1α,2β,5α) were observed to be prominent. Hydroquinone acetate (1,4-dihydroxybenzene acetate) have been identified from different species in Asterales, Ericales and Lamiales taxa, in brown and green algae and in bacteria. It has also been seen in higher marine invertebrate like ascidians, cnidarias and sponges (Roas *et al.*, 2022). Derivative of this compound are depigmentation agent, use in the treatment of dyschromia (Schwartz *et al*., 2023). Ultraviolet B irradiated Arbutin and DeoxyArbutin (glucoside derivative of hydroquinone) have been shown to have high cytotoxicity for fibroblast cells, with the activation of caspase-3 by irradiation of DeoxyArbutin in Detriot 551 cells, these were observed to correlate with hydroquinone (Chang *et al*., 2017). Hydroquinone has also been reported to induce a dose response inhibition of cell growth and DNA damage which was associated with increased oxidative stress. It was further shown that it effect was more pronounced after 24 hours (LC_{50}=33 µm) compared to an hour of exposure (LC_{50}=59 µm) (Peng *et al*., 2013).

The HPLC analysis revealed the presence of wide array of phenolic compounds which may have self-protective effect on the plant and numerous pharmacological potentials including cytotoxicity (Nejat and Mantri, 2017). Most of the reported underlying mechanism of action of cytotoxicity from plant extracts involves the activation of apoptosis, inhibition of angiogenesis and inhibition of pro-inflammatory signaling (Gao *et al*., 2011). Document evidence have shown that antioxidants (phenolics, phenolic acids and flavonoids) are effective cytotoxic agents towards different cancerous cells (Ganesan and Xu, 2017). Apart from their antioxidant property, they also possess anti-inflammatory action. These combined properties have been shown to aid in the treatment or management of degenerative diseases, characterized by oxidative stress and inflammatory responses (Lopez-Corona *et al.*, 2022). Individually, ferulic acid has been shown to induce arrest at the G0/G1 phase in HeLa and CaSki cervical carcinoma cells. Reduces the expression of cyclin D1 and E, and induces the expression of p52 and p21 (Gupta *et al*., 2021; Moghadam *et al.*, 2021). Ellagic acid has been shown to activate AMPK and inhibit HIF-1α in lung cancer (Heidarian *et al*., 2016).

Methanol extract of *E. praetermissa* Milne-Redhead caused cytotoxicity in *R. raninus* within 90 min at a dose of 320µg/mL, while ethylacetate fraction at similar dose produced cytotoxic effect within 60 min of its exposure, implying that the fraction contains polar compounds that could be responsible for this effect. At 160µg/mL, methanol extract showed cytotoxic effect within 150 min, while the fractions (dichloromethane and ethylacetate) showed activity within 90 min. Since fractionation of the methanol extract, enables separation of the non-polar compounds from polar compounds, thus these partially separated compounds can now have direct contact with the cells of *R. raninus*, and produced effect within a shorter time. Furthermore, it took 24 hours for the methanol extract to cause 100 % mortality at 80µg/mL, while the fractions caused 100 % mortality within 120 min., showing a reduction in the activity of the extract and fractions. Only dichloromethane fraction showed activity at 40µg/mL within 24 h, while methanol extract and ethylacetate fraction produced no effect. No mortality was seen at 20µg/mL, this is similar to the effect produced by the control. The exact mechanism of action of the extract and fractions may not be known, literature search have revealed four groups of compounds with different mechanism of action;



hydroquinone acetate



Bicyclo[3.1.1]heptane,2,6,6-trimethyl-(1a,2ß,5a)

Cytotoxicity is caused by exogenous chemical which can result in cell death; this effect is considered to be dose dependent and species specific. Examples of cyto-toxicant include natural plant extract and pharmaceutical drugs. One of the well-established mechanisms involves the excessive production of nitric oxide (NO), reactive oxidative species and ultimately oxidative stress occurs (Zhang, 2018). Also cytotoxic agents could induce or cause the release of substances that damage DNA in the nucleus of the cell and finally apoptosis (Hu *et al*., 2005).

Conclusion

The leaf of *E. praetermissa* Milne-Redhead have been shown to be rich in different classes of phytochemicals, some were identified and quantified from the methanol extract from GC/MS and HPLC analyses. Many of these identified phytochemicals have documented pharmacological activities. Ferulic acid, ellagic acid, hydroquinone acetate and bicyclo(3.1.1] heptane-2,6,6-trimetyl (1a,2β,5a) were identified from the leaves of *E. praetermissa* Milne-Redhead with reported cytotoxic property. This property may justify its use in the treatment of tumor and possible anticancer agent, thus there is need for further studies to isolate these compounds and evaluate against different cancer cell lines.

Conflict of Interest
The authors declare that there is no conflict of interest associated with this study.

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