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PHYSICOCHEMICAL STUDY OF *MELALEUCA LEUCADENDRA* ESSENTIAL OIL

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ABSTRACT

Essential oils can be derived from both medicinal and aromatic plants. It is important to note that all plants have the ability to produce volatile substances, but only in trace amounts, with the exception of fragrant plants. The initial step in exploiting the chemical potential of aromatic plants is the extraction of specific compounds. Essential oils are obtained by various extraction methods, including hydro-distillation, the method chosen to extract our oil, which consists of immersing the raw material in a water bath and bringing the whole mixture to a boil. This study presents the extraction of essential oil from *Melaleuca leucadendra* leaves and the characterization of its chemical composition. A percentage of 1.004% was obtained after extraction and, to determine the chemical composition, the essential oil was analyzed by GC-MS and 40 chemical constituents were identified. The oil was mainly characterized by the presence of 1,8-cineole with 15.49%, followed by viridiflorol with 12.49%, trans-nerolidol with 10.96%, and alpha-terpineol with 9.77%.

KEYWORDS: *Melaleuca leucadendra*, essential oils, characterization, GC-MS.

INTRODUCTION

Since ancient times, plants have always been used by local populations for food, perfumery with aromatic plants, makeup, but above all for health, thanks to the active ingredients of the secondary metabolites they contain, which enable organisms to defend themselves against external and internal aggressions. Traditional populations had no idea what could be behind the therapeutic properties of the plants they used, but with the development of science, these properties have been justified through phytochemical studies. It is thanks to traditional medicine that phytotherapy came into being[1], and an integral part of this, using the concept of essential oils, is aromatherapy, which is a natural therapy that uses plant essential oils internally and externally to cure or prevent diseases. Essential oils were practically discovered in the Middle Ages as a result of the first distillations and later thanks to scientific progress and, in particular, the advent of chemistry.

Essential oils are included in the range of products offered to people who want to reconnect with a more natural way of caring for themselves. In addition to aromatherapy, essential oils are also used in perfumery, cosmetics, and pharmacy. These multiple uses are due to the various recognized biological properties they contain, which can be explained by the biochemical composition of these oils. Essential oils are defined as products with a generally complex composition containing the volatile principles of plants.[2] In other words, they are oily substances without fat, more or less fluid, even volatile resinoids, fragrant, often colored or colorless, sensitive to heat, also known as liquid aromatic organic substances obtained from plant raw materials. They are found naturally in different parts of plant species (leaves, seeds, flowers, stems, roots, bark, fruit, etc.).

They are obtained through various extraction processes, namely: steam distillation, hydro-distillation, cold pressing, organic solvent extraction, microwave-assisted extraction, and supercritical fluid extraction. Most plant species contain essential oils, but in very small quantities. Only so-called aromatic plants contain sufficient quantities.[3] Among the various families of aromatic plants is the Myrtaceae family, to which the species *Melaleuca leucadendra* belongs and whose essential oil is the subject of our study. The aim of our study is to provide you with a bibliography on the species, discussing its botany, pharmacopoeia, use in traditional medicine, and application in other sectors, then to present the results we obtained following the extraction of essential oil from the leaves of *Melaleuca leucadendra*, and finally to discuss the yield and chemical composition of our oil as analyzed by GC-MS.

I. Presentation of the *Melaleuca leucadendra* plant

1. Ethnobotanical study

Plants of the Melaleuca genus belong to the Myrtaceae family and are better known as "tea trees." They comprise approximately 130 genera and around 3,800-5,800 species found in tropical and subtropical regions. The common name Melaleuca comes from the Greek "melas," meaning black or dark, and "leucon," meaning white, referring to the black branches and trunk of the original species called *Melaleuca leucadendra*. Melaleuca species range from woody shrubs with multiple trunks to very large, single-stemmed trees that produce wood. The different species of the Melaleuca genus are found almost everywhere in the world, particularly in Tasmania (Australia), tropical America, Indonesia, Papua New Guinea, and southern Asia, in open forests, wooded areas, or shrubby areas along riverbanks and swamps.[4] Melaleuca are trees and mostly shrubs, most of which do not reach 10 m in height, with nearly 40 species not exceeding 1 m in height.[3]

The species *Melaleuca leucadendra* is sometimes a large tree (20 to 40 m tall, with a diameter of up to 1.5 m) and sometimes a dwarf shrub. In the tree form, the branches are usually pendulous, sometimes erect if the tree is leaning or lying down. The leathery leaves are reminiscent of the phyllodes of Australian Acacia.[2]

Photo 1: Leaves of *Melaleuca leucadendra*.

2. Pharmaceutical

The use of medicinal plants in medicine has been practiced for thousands of years and is used by most peoples around the world. *Melaleuca leucadendra* is cultivated not only for its ornamental value and economic importance, but also for its medicinal properties. Different parts of the species, including its essential oil, are used in traditional medicine.[4] *Melaleuca leucadendra* essential oil has a wide range of uses in conventional medicine, including as an antiseptic, antispasmodic, insect repellent, antineuralgic, termite repellent, expectorant for throat applications, and antiviral. It has been used in aromatherapy to treat neurogenic pain, provide temporary relief from muscle pain, treat susceptibility to infections, burns, and as a tonic.[6] There are also other indications for cajeput essential oil, such as its use for stomach aches (as an ointment), muscle and joint pain in rheumatic diseases, sciatica, low back pain, and disc dislocations. It accelerates and facilitates wound healing, helping to completely revitalize the skin while reducing bacteria on the wound, and therefore, it can be inferred that the oil has bactericidal effects.[7]

Melaleuca leucadendra essential oil is used in the food industry as an aromatic ingredient and food supplement.[8] In the cosmetics industry, tea tree oil is also used to manufacture many plant-based personal care and household products such as liniments, ointments, perfumes, soaps, shampoos, shower gels, insect repellents, etc.[2]

II. MATERIALS AND METHODS

1. Plant Materials

The leaves of *Melaleuca leucadendra* were harvested at the Hann/Maristes National Zoological Park (Dakar, Senegal). The date and time of harvest were February 2, 2025, between 6:30 a.m. and 7:20 a.m. They were then dried indoors at room temperature from February 2 to February 10, 2025.

2. Extraction

The method used to obtain our essential oil is hydrodistillation, which is generally the most commonly used process for extracting essential oils. The principle consists of mixing a solvent (water) with plant material (ML leaves) and bringing the mixture to a boil.

We mixed 50.76g of *Melaleuca leucadendra* leaves with 850mL of water in a 1L flask. Once assembled, the flask is placed on the hotplate. The mixture is brought to a boil at 100 degrees. The mixture of water vapor and volatile compounds passed through a cooling tube to be condensed. After condensation, the liquid was collected in a separating funnel and left to settle so that the essential oil could be separated and collected in a pill bottle. The pill bottle was hermetically sealed with parafilm to prevent volatilization, and finally the oil contained in the pill bottle was stored in the refrigerator until further chemical analysis and biological activity tests could be performed.

Photo 2: Extraction of essential oil by hydro-distillation.

2. Identification of chemical composition

The various components of *Melaleuca leucadendra* essential oil were analyzed using gas chromatography coupled with mass spectrometry (GC-MS). Measuring retention times does not provide formal proof of the nature of the compounds. The identification of the compounds is confirmed by the GC/MS coupling technique.

III. RESULTS AND DISCUSSIONS

1. Results

1.1. Extraction percentage

The percentage of essential oil extraction is defined as the ratio between the mass of essential oil obtained after extraction and the mass of the raw plant material used (dried leaves). The result is expressed as a percentage:

$$EP = \frac{mass\ EO}{mass\ MPM} * 100$$

- EP: extraction percentage
- Mass EO: mass of essential oil
- Mass MPM: mass of plant material

Table 1: Percentage of essential oil extraction.

Mass MPM (g)	Masse EO (g)	EP
50,76g	0,51g	1,004

1.2. Chemical composition

Essential oils are a source of raw materials in several fields of activity such as agri-food, pharmacology, cosmetics, and perfumery, which means that it is necessary and important to know their chemical compositions. The results are shown in the table below:

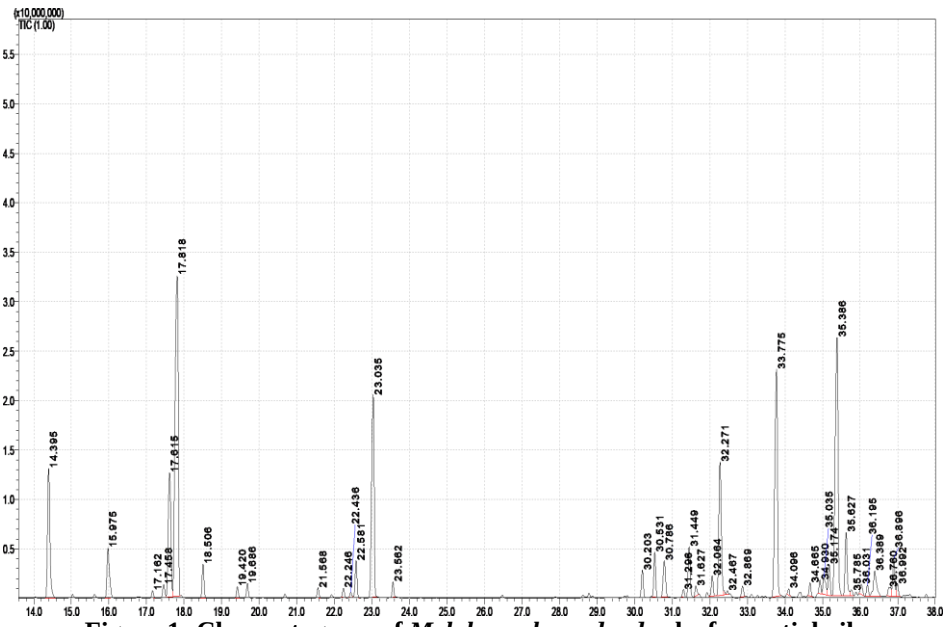

Figure 1: Chromatogram of *Melaleuca leucadendra* leaf essential oil.

Table 2: Results of the chemical composition of *Melaleuca leucadendra* essential oil.

Peak	Name	R. Time	I. Time	F. Time	Area	Area%	Height	Height%
1	2-Pinene	14.395	14.305	14.580	58657344	5.90	13116770	6.26
2	Beta-Pinene	15.975	15.895	16.115	23711564	2.38	5051330	2.41
3	1,3-Cyclohexadiene, 1-methyl-4-(1-methylet	17.162	17.110	17.235	2404813	0,24	723436	0.35

4	O-Cymene	17.458	17.395	17.520	4941508	0.50	1285335	0.61
5	D-Limonene	17.615	17.520	17.695	62200145	6.26	12583384	6.01
6	Eucalyptol	17.818	17.695	17.900	192484192	19.36	32432353	15.49
7	Gamma-Terpinene	18.506	18.435	18.590	11836930	1.19	3432535	1.64
8	4-Isopropylidene-1-cyclohexene	19.420	19.360	19.490	3801953	0.38	1148706	0.55
9	Linalol	19.686	19.630	19.760	4541058	0.46	1436561	0.69
10	dl-Isopulegol	21.568	21.510	21.635	3227041	0.32	1006065	0.48
11	Cyclohexanemethanol, alpha.,. alpha. -dimethyle	22.246	22.190	22.315	3079841	0.31	948836	0.45
12	Borneol	22.436	22.390	22.490	1683915	0.17	492836	0.24
13	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl	22.581	22.490	22.665	13247772	1.33	3758740	1.79
14	Alpha-terpineol	23.035	22.915	23.140	96678423	9.72	20453273	9.77
15	6-Octen-1-ol, 3,7-dimethyl-	23.562	23.505	23.630	4735453	0.48	1550425	0.74
16	Caryophyllene	30.203	30.130	30.280	9981740	1.00	2773008	1.32
17	Cis- beta-Farnesene	30.531	30.460	30.610	15363588	1.55	4557644	2.18
18	1,1,4,7-Tetramethyl-1a,2,3,4,6,7,7a,7b-octahy	30.786	30.700	30.885	16689278	1.68	3690366	1.76
19	Alpha Humulene	31.296	31.240	31.365	2658761	0.27	778773	0.37
20	Neoalloocimene	31.449	31.370	31.560	19756275	1.99	5132795	2.45
21	Trans-alpha. -Bergamotene	31.627	31.565	31.570	3682289	0.37	1025114	0.49
22	Alpha-Farnesene	32.064	31.975	32.135	6967356	0.70	2089693	1.00
23	1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-o	32.271	32.135	32.370	62002028	6.24	13502684	6.45
24	Naphtalene, 1,2,3,4,4a,5,6,8a-octahydro-4a	32.467	32.370	32.510	1903198	0.19	387860	0.19
25	Beta-Cadinene	32.869	32.805	32.940	4094025	0.41	1081665	0.52
26	Trans-Nerolidol	33.775	33.640	33.900	112892799	11.35	22956308	10.96
27	3,5,9-Trimethyl-deca-2,4,8-trien-1-ol	34.096	34.050	34.160	1833935	0.18	640243	0.31
28	4aH-Cycloprop[e]azulen-4a-ol, decahydro-1,	34.665	34.605	34.745	4539818	0.46	1344298	0.64
29	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa [e	34.930	34.870	34.980	4460623	0.45	1380344	0.66
30	Caryophyllene oxide	35.035	34.980	35.120	9177225	0.92	1703612	0.81
31	Guaiol	35.174	35.120	35.250	11107856	1.12	3160038	1.51
32	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,	35.386	35.250	35.520	143130994	14.40	26159543	12.49
33	Ledol	35.627	35.520	35.725	25723074	2.59	6435057	3.07
34	(1R,3E,7E,11R) -1,5,5,8-Tetramethyl-12-oxab	35.785	35.725	35.835	2418450	0.24	500489	0.24
35	2-((4aS,8R,8aR) -4a,8-Dimethyl-3,4,4a,5,6,7,	36.031	35.970	36.120	4421432	0.44	1055055	0.50
36	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octah	36.195	36.130	36.265	4301420	0.43	1127006	0.54
37	Tau-cadinol	36.389	36.265	36.555	15501028	1,56	2570239	1.23
38	Tau-muurolol	36.760	36.705	36.840	5337658	0.54	857796	0.41
39	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-oct	36.896	36.840	36.955	14710181	1,48	3839935	1.83
40	2,6,10,10-Tetramethylbicyclo [7.2.0] undeca-1,	36.992	36.955	37.070	4345546	0.44	1272159	0.61
					994232529	100.00	209442309	100.00

2. DISCUSSIONS

2.1. Extraction percentage

Hydro-distillation extraction of *Melaleuca leucadendra* leaves yielded an extraction percentage of 1.004% in essential oil. Compared to previous studies, this can be considered a satisfactory yield. Few results on the extraction percentages of *Melaleuca leucadendra* leaves by hydro-distillation have yielded essential oil contents exceeding 1%. Mbodj et al. conducted a recent study in 2024 on the leaves of the species and obtained an exceptional yield of 1.75% essential oil[9], given that plants generally produce minimal amounts of essential oil, which explains their low yields of less than 2% in most cases. Other authors who have obtained yields above 1% include Padalia et al. in 2015, who obtained a yield of 1.20% essential oil extracted from the leaves of *Melaleuca leucadendra*.[10]

Two groups of researchers obtained returns of 0.8% and 0.75% respectively, close to 1%. These were L. Monzote et al in 2020[11] and Jing Zhang et al in 2019.[12] Milena MC da Silva et al[13] and Saima Siddique et al[14] obtained low yields of 0.28% and 0.42% in essential oil in 2020, compared to the yields mentioned above. Recently, in 2024, Phuong Ha Tran et al. obtained an extremely low yield of 0.31%[15] compared to our yield.

2.2. Chemical composition

GC-MS analysis of the essential oil extracted from *Melaleuca leucadendra* leaves revealed the presence of 40 chemical constituents representing 99.54% of the total amount of essential oil. The different subfamilies to which these chemical components belong are: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes. The main constituents of the essential oil are: the oxygenated monoterpene 1,8-cineole or

eucalyptol (15.49%), which is the major constituent in the essential oil, followed by the oxygenated sesquiterpene viridiflorol (12.49%) or 1H-Cycloprop[e]azulen-4-ol, decahydro-1,1, followed by trans-nerolidol (10.96%), which is also an oxygenated sesquiterpene, and finally α-terpineol (9.77%), an oxygenated monoterpene. Some of the remaining constituents were present in small quantities and others in trace amounts.

In terms of chemical constituents, 1,8-cineole is the main compound and is one of the oxygenated monoterpenes. However, in terms of subfamilies, oxygenated sesquiterpenes dominate the essential oil, representing 35.81% of the total composition, followed by oxygenated monoterpenes with 29.17%, then monoterpene hydrocarbons (17.83%) and sesquiterpene hydrocarbons (16.73%). All the constituents found in the oil have been reported in previous studies, but it should be noted that the essential oil content and predominant chemotypes varied from one study to another, from one country to another, and from one harvesting area to another.

In 2024, the results of GC-MS analyses of the composition of essential oil from *Melaleuca leucadendra* leaves collected in Vietnam showed a predominance of the following compounds: β-caryophyllene (14.11%), γ-terpinene (12.94%), terpinolene (11.77%), khusimone (9.87%), α-humulene (8.54%), α-pinene (7.69%), lene oxide (7.22%) and p-cymene (5.38%) according to Tran et al. They found 19 compounds in their essential oil, which represented 95.93% of the total composition of the oil.[16] In 2012, Pujiarti et al obtained the following main constituents of the oil from leaves collected in Java, Indonesia: 1,8-cineole (49.22–55.04%), α-terpineol (8.79–10.70%), γ-limonene (5.58–6.39%), and β-caryophyllene (5.03–7.64%).[17]

The oil extracted from the leaves of the species harvested in India was dominated mainly by sesquiterpenoids (95.34%), consisting of 93.50% oxygenated sesquiterpenes and 1.84% sesquiterpene hydrocarbons. (E)-nerolidol (90.85%), caryophyllene (1.52%), caryophyllene oxide (0.99%), and globulol (0.70%) were the main sesquiterpenoids in the leaf oil.[10] Pino et al. in 2010 found that oil from leaves harvested in Cuba was rich in compounds such as: 1,8-cineole (43.0%), viridiflorol (24.2%), terpineol (7.0%), α-pinene (5.3%) and limonene (4.8%).[5] Pino and Padaliaa identified another component in minimal quantities in the oil from the leaves of *Melaleuca leucadendra*, namely a-terpinyl acetate, with respective percentages of 1.6% and 0.53%.[5-10] Studies conducted in Brazil in 2007 by Silva et al. and in Senegal in 2022 by Diallo et al. showed that the main component of *Melaleuca leucadendra* essential oil was methyl eugenol.[18-19] Monzote et al. in 2020 reported 1,8-cineole as the main constituent among the 45 constituents detected in their essential oil from leaves collected in Havana, Cuba.[11]

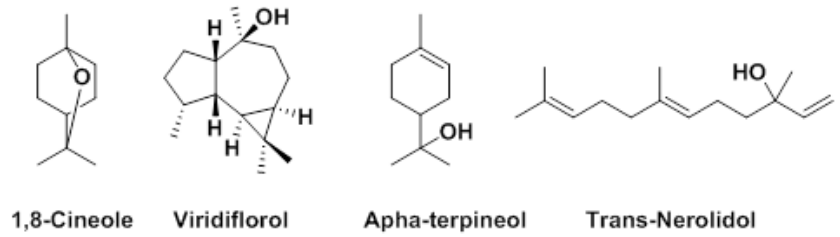

Figure 2: Main constituents or major constituents of the essential oil from *Melaleuca leucadendra* leaves.

2.3. Pharmaceutical and cosmetic benefits of the oil's composition

In the pharmaceutical and cosmetic industries, essential oils are attractive options or sources of natural (or green) alternatives to synthetic preservatives.

2.3.1. Pharmaceutical interest

Due to the many interesting biological properties of essential oils, they represent an alternative to the synthetic molecules or substances used in the pharmaceutical industry. Essential oils of the Melaleuca genus are used as active ingredients in the pharmaceutical industry, and various health products are manufactured from this essential oil.[20] In Europe, the European Centre for Disease Prevention and Control (ECDC) has recorded a high death rate caused by antibiotic-resistant bacteria. Thus, ultra-resistant superbugs represent an emergency and a global threat that the WHO must address to find immediate solutions. The WHO has therefore devoted much of its research and efforts to exporting traditional knowledge of herbal medicine, with the aim of offering natural molecules as alternatives to synthetic antibiotics to combat antibiotic resistance. These natural substances, including essential oils, have become the new antimicrobial agents and are a promising source due to their biodiversity, effectiveness, and natural origin.

The two main classes of bioactive compounds present in essential oils are terpenoids and phenylpropanoids. The latter have a very broad spectrum of action and are effective against a large number of bacteria, including the most resistant ones.[21] The components of *Melaleuca leucadendra* essential oil, such as 1,8-cineole, α-pinene, linalool, and α-terpineol, are known for their bacteriostatic function. Monoterpenes, namely β-pinene and limonene, have an antiviral effect, particularly against the Herpes simplex virus type 1.[22] The

acaricidal properties of 1,8-cineole and terpinene-4-ol have also been well proven. Studies have shown that α-terpineol can act as an anticancer agent and is also an antidepressant.[23-24] Trans-nerolidol is a sesquiterpene alcohol that has various properties such as antitumor, repellent or insecticidal, anticancer, antiulcer, antinociceptive, anti-inflammatory, antioxidant, antifungal, and antimicrobial activities. All these properties make trans-nerolidol a promising candidate for use in the manufacture of drugs in the therapeutic and pharmaceutical fields.[25]

Volatile bioactive compounds are also used for their neuroprotective, hypoglycemic, nematicidal, virucidal, and enzyme-inhibiting properties. In oncology, 60% of the 246 anticancer agents discovered between 1940 and 2014 come from natural plant substances. More than half of the drugs approved by the Food and Drug Administration (FDA) are derived from natural products and their derivatives. To facilitate and accelerate the discovery of new natural and effective drugs, various databases have been created.[20]

2.3.2. Cosmetic interest

Thanks to the many biological properties and pleasant scent of terpenoids, particularly monoterpenes and sesquiterpenes, these volatile organic compounds are widely used in the cosmetics and perfume industries.[25] Studies have shown that caryophyllene, a sesquiterpene compound, is present in more than 33% of cosmetic products tested on the Dutch market and is also found in more than 45% of deodorants sold on the European market.[26] Caryophyllene has powerful anti-inflammatory and healing properties for the skin.[27]

In the field of cosmetics, *Melaleuca leucadendra* essential oil is used to manufacture numerous plant-based personal care and household products such as liniments, ointments, perfumes, soaps, shampoos, shower gels, insect repellents, etc.[3] The essential oil of the leucadendra species has demonstrated antioxidant properties that have been scientifically proven by numerous free radical scavenging tests. These anti-radical activities are due in part to the presence of phenolic compounds in the plant species, but also to terpenic molecules such as 1,8-cineole, α-terpineol, α-pinene, limonene, globulol, and guaiaol contained in the essential oil of Melaleuca leucadendra, making it an essential ingredient for cosmeceutical product formulators thanks to its natural effectiveness.[28]

CONCLUSION

This study involved extracting and analyzing the chemical composition of the essential oil from *Melaleuca leucadendra* leaves. The essential oil analyzed by gas chromatography coupled with mass spectrometry was characterized by a predominance of oxygenated sesquiterpenes, with 1,8-cineole as the major chemical constituent. Through this study, we were able to compare our results with those of previous work on the chemical composition of essential oil from the leaves of the leucadendra species, and each time we saw that the chemical composition and predominance of chemotypes varied from one study to another depending on the geographical location of the area.

Based on the different molecules isolated from the essential oil and other organic extracts from the leaves of *Melaleuca leucadendra*, biological tests will be carried out for the continuation of our research. The main constituents of our essential oil have scientifically proven biological activities. These include 1,8-cineole, which has anti-inflammatory, antibacterial, and nematicidal properties, and viridiflorol, trans-nerolidol, and α-terpineol, all of which have anticancer activities.

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