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Chemical composition, antioxidant and antimicrobial activities of essential oils from *emilia praetermissa* Milne-Redh from Cote D'Ivoire: A comparative analysis of hydrodistillation and steam drive methods

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Abstract

This study aims to enhance the value of Ivorian aromatic plant resources through a comparative analysis of the influence of extraction methods on the chemical composition and biological properties of essential oils. The chemical composition of the essential oil from the aerial parts of *Emilia praetermissa* Milne-Redh (Asteraceae) was carried out for the first time by gas chromatography coupled to mass spectrometry (GC-MS) with calculation of retention indices. Antioxidant and antimicrobial activities were evaluated by spectrophotometry and microdilution methods, respectively.

Two extraction processes were compared: hydrodistillation (HD) and steam distillation (SD). The essential oil obtained by HD has a pale yellow color compared to that obtained by SD which is yellow. The extraction yield using HD ($0.06 \pm 0.02\%$) was significantly higher than that obtained using SD ($0.04 \pm 0.02\%$).

Phytochemical analysis revealed that the essential oils were predominantly composed of oxygenated compounds (33.11-49.63%), followed by alkenes (23.66-25.87%), sesquiterpenes (20.23-21.63%), and monoterpenes (3.34-14.2%). The major compound identified were caryophyllene oxide (24.02-27.74%) and 1-undecene (23.66-25.58%), regardless of the extraction method. Both essential oils demonstrate remarkable antioxidant potential, with RC_{50} values of 0.014 ± 0.01 mg/mL (EO-SD) and 0.026 ± 0.01 mg/mL (EO-HD). Antimicrobial activity assessment revealed efficacy against the majority of bacterial and fungal strains tested, with minimum inhibitory concentrations (MIC) of 3.75 mg/mL.

These results highlight quantitative and qualitative variations in the chemical composition of essential oils depending on the extraction process, confirming the importance of methodological choice for optimizing biological properties.

Keywords: *Emilia praetermissa*, essential oil, hydrodistillation, steam distillation

1. Introduction

Côte d'Ivoire, rich in its vast and abundant floral biodiversity, benefits significantly from its endogenous traditional medicine. In sub-Saharan Africa, the use of aromatic and medicinal plants is a common practice, valued for their intrinsic properties [1-2]. Among these plant species of interest is *Emilia praetermissa*. Initially identified in Sierra Leone and Nigeria [3], *E. praetermissa* has since been located in several other West African countries, including Cameroon, Ghana, Guinea, and Liberia [4-6], confirming its presence also in Côte d'Ivoire. Morphologically, *E. praetermissa* shows strong similarity to *E. fosbergii*, an invasive species naturalized in the Neotropics and Pacific regions [5]. It is an annual herb that can reach up to 140 cm in height. Its stems, erect or ascending, which can be simple or branched from the base, are glabrous and measure between 0.6 and 2 cm long, with internodes exceeding 9 cm [7]. These botanical characteristics are essential for its identification and study in pharmacognosy. In Ivory Coast, an ethnobotanical survey reveals that *E. praetermissa* is traditionally used in the treatment of gastric pain, malaria, childhood pathologies and as a support during pregnancy [8]. The studies available in the literature mainly focus on the chemical and biological properties of extracts obtained by organic solvents. The acute toxicity assessment indicates an LD_{50} greater than 2.0 g/kg in rats. Regarding subacute toxicity, the results suggest that prolonged administration of high doses can induce hepatotoxic, pneumotoxic and nephrotoxic effects [9].

Furthermore, investigations suggest a potential protective effect of the extract against the development of atherosclerosis and coronary pathologies, as well as against dyslipidemias associated with diabetes mellitus, hypertension and obesity. These data indicate that the aqueous extract of *E. praetermissa* could be an effective therapeutic adjunct in patients with hyperlipidemia^[10]. The antimicrobial activity of methanolic, hot aqueous, and cold aqueous extracts of *E. praetermissa* leaves was evaluated against microorganisms isolated from patients with otitis media using the agar well diffusion method. All three extracts tested demonstrated inhibitory activity comparable to that of standard commercial antibiotics^[11].

To date, no study has been devoted to the analysis of *Emilia praetermissa* essential oils, leaving a gap in the knowledge of its phytochemical and biological properties. It is in this context that the present study was initiated, with the main objectives of characterizing and comparing the chemical composition of essential oils obtained by hydrodistillation and steam distillation, and then evaluating their antioxidant and antibacterial activities.

2. Materials and Methods

2.1. Plant Materials

The aerial parts (leafy stems and flowers) were harvested in July in the commune of Cocody, located in Abidjan, Côte d'Ivoire (coordinates: 5° 21' 23" N, 3° 58' 57" W). Plant identification was carried out at the National Floristic Center (CNF) in Abidjan, using a herbarium specimen under number UCJ003601.

2.2. EO Extractions

2.2.1 Hydrodistillation

The extraction was carried out using a Clevenger apparatus combined with a 10 L pressure cooker. The plant material was placed on the pressure cooker's rack, previously filled with water below the rack level. The system was heated using a hotplate until boiling. The water vapor, laden with volatile aromatic compounds, passed through the Clevenger glass cylinder before being condensed by a condenser. After 4 hours of extraction, the essential oil was separated from the water by natural decantation. To remove residual moisture, the EO was dried with anhydrous magnesium sulfate (MgSO₄), then stored in opaque bottles at 4 °C in a refrigerator.

2.2.2 Steam distillation

The essential oil was extracted by steam distillation using a four-compartment stainless steel system. The 60-liter boiler produces steam, which is conveyed to a large cylindrical tank (100 cm high, 51 cm in diameter) containing four stacked screens containing the plant material. The steam, laden with aromatic compounds, then passes through a condenser of similar dimensions (100 cm x 41 cm) before being collected in a separator. The resulting essential oil is immediately packaged in opaque bottles protected by aluminum foil and then stored at 4 °C in a freezer to preserve its quality. This method allows for efficient extraction while minimizing the degradation of volatile compounds.

2.3 EO Analysis by GC-MS

The analysis of EOs diluted in dichloromethane (1:100) was performed on a GC chromatograph (7890A, Agilent Technologies) coupled with a mass spectrometer (5975C,

Agilent Technologies). A sample of EO (1 µl) was injected into an HP-5MS capillary column at 250 °C. The oven temperature was programmed at 40 °C for 5 min, then at 2 °C/min for 15 min up to 250 °C, with a flow rate of 10 °C/min up to 300 °C. Helium (carrier gas) flow rate 1mL/min, MS detector temperature 280 °C, detector voltage 1.4 kV, ion mass/charge ratio scan range 40 to 500. Retention indices were calculated from retention times^[12-14].

$$RI = 100 \left[n + \frac{t_R(C_i) - t_R(C_n)}{t_R(C_{n+1}) - t_R(C_n)} \right]$$

Compound identification was performed by comparing the calculated retention indices and obtained mass spectra with those in the National Institute of Standards and Technology (NIST) database^[15], the National Institute of Health Chemistry Database (PubChem-online)^[16], and the literature [17-18].

2.4 In vitro evaluation of antioxidant activity using the DPPH test

A 0.03 mg/mL stock solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was prepared by solubilizing the reagent in absolute methanol. The essential oil under test (20 mg) was dissolved in 5 mL of methanol and then diluted to obtain concentrations ranging from 0.0625 to 4 mg/mL. For the test, 2.5 mL of each dilution was mixed with 1 mL of DPPH solution in sterile tubes. After shaking, the tubes were incubated in the dark for 30 minutes. Absorbance was measured at 517 nm using a spectrophotometer (AQUA LYTIC AL800), using a mixture of 2.5 mL of methanol and 1 mL of DPPH solution as a blank. Vitamin C was used as a positive control, prepared under the same experimental conditions in Blois.^[19] The 50% reducing concentration (RC₅₀) was determined by graphical interpolation, according to the method described by Tanoh *et al.* and then Etekpó *et al.* [20-21].

2.5 Evaluation of in vitro antimicrobial activities

2.5.1 Determination of inhibition zone diameters

The reference strains used in this study of susceptibility to various Essential Oil (EO) concentrations were provided by the laboratory of the Swiss Center for Scientific Research in Côte d'Ivoire. These strains include: *Staphylococcus aureus* CIP 4.8, *Staphylococcus epidermidis* CIP.53124, *Salmonella typhimurium* SO 66, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Candida tropicalis* ATCC 13803 and *Candida glabrata* ATCC 66032. The antibiotics Gentamycin and Amphotericin B served as positive controls, their zones of inhibition being measured under identical conditions.

The evaluation of the antimicrobial activity of the different concentrations of EO against each strain was carried out according to the method of Berghe and Vlietinck^[22]. The experimental protocol consisted of inoculating the surface of Mueller-Hinton medium poured into Petri dishes with 1 ml of inoculum from 18-20 hour cultures (10⁵-10⁶ CFU/ml). After waiting 15 minutes, wells were dug using Pasteur pipettes, then sealed at the bottom with a drop of MH agar to prevent the diffusion of oils under the agar. Each well then received 50 µl of EO at the tested dilutions. After a diffusion phase, the cultures were incubated at 37 °C for 24 hours. The diameters of the inhibition zones were determined using a caliper.

2.5.2 Determination of Minimum Inhibitory and Bactericidal Concentrations

A volume of 0.1 ml of broth is inoculated into 10 ml of Mueller-Hinton (MH) broth for strains of *Escherichia coli*, *Salmonella*, *Bacillus*, *Klebsiella*, and *Candida*, while 0.3 ml is used for *Staphylococcus aureus* and *S. epidermidis*. These cultures are incubated at 37 °C for 3 to 5 hours until a slight opalescence is obtained, corresponding to approximately 5×10^7 bacteria/ml. Subsequently, 1 ml of these broths is added to 10 ml of MH broth previously heated to 37 °C to constitute the inoculum. The microplate dilution protocol is carried out as follows: 100 µl of the essential oil solution is placed in column 12, 50 µl of MH broth in columns 11 to 2, and 100 µl of MH broth in column 1. A series of half-fold dilutions is carried out from column 12 to column 3 by transferring 50 µl at each step. The inoculum (50 µl) is then distributed in each well of columns 12 to 2. After incubation at 37 °C for 18 hours, visual reading allows the determination of the minimum inhibitory concentration (MIC), corresponding to the last dilution showing no turbidity. Amphotericin B and Gentamicin are used as reference antibiotics.

3 Results and Discussion

3.1 Color and Yields

The essential oils obtained were yellow. Extraction yields were 0.06% for hydrodistillation and 0.04% for steam distillation. These yield variations can be attributed to several parameters, including the distillation technique used, the

harvest period, and the geographical area of collection. The higher yield obtained by hydrodistillation compared to steam distillation could be explained by the characteristics of the recovery system. Indeed, the larger contact surface area of the settling funnel promotes the dispersion of the essential oil, making its recovery more difficult and thus reducing the final yield. Taha *et al.* [23] demonstrated the superiority of Clevenger distillation (a form of hydrodistillation) in the extraction of essential oil from the aerial parts of *Rosmarinus officinalis*. The results indicate yields of 2.4% by Clevenger, 2.2% by industrial distillation (steam distillation), and 2.1% by artisanal distillation.

3.2 Chemical Composition

Table 1 shows the results of the GC-MS chemical analysis of the essential oil from the aerial parts of *E. praetermissa*, obtained by hydrodistillation. Thirty-two (32) identified compounds represent 93.41% of the total composition. This EO is mainly composed of oxygenated compounds (33.11%), followed by alkenes (25.87%), sesquiterpene hydrocarbons (20.23%), and monoterpene hydrocarbons (14.2%). The main constituents are the alkene 1-undecene (25.58%), caryophyllene oxide (24.02%), and caryophyllene (8.34%). Also present in smaller proportions are compounds such as α -pinene (3.35%), β -myrcene (5.16%), cyclosativene (3.16%) and 6-epi-cubenol (3.31%). On the other hand, analysis of the EO obtained by steam distillation revealed 18 compounds, covering 98.26% of the total composition.

Table 1: Chemical composition of *E. praetermissa* aerial parts EO

N°	compounds	RT (min.)	RI	m/z	% Total
1	α -pinene	12.63	925	136	3.35
2	camphene	13.52	938	136	0.13
3	β -pinene	15.36	967	136	2.74
4	β -myrcene	16.74	989	136	5.16
5	D-limonene	19.10	1023	136	2.82
6	1-undecene	23.90	1090	154	25.58
7	(Z)-3-undecene	24.48	1098	154	0.29
8	bornyl acetate	37.11	1280	196	0.95
9	cyclosativene	42.16	1358	204	3.16
10	longipinene	42.52	1363	204	0.37
11	copaene	43.55	1379	204	0.20
12	β -bourbonene	43.94	1385	204	1.28
13	β -elemene	44.72	1397	204	0.18
14	caryophyllene	45.43	1409	204	8.34
15	α -muurolene	47.50	1443	204	0.93
16	α -humulene	48.18	1454	204	0.16
17	germacrene D	49.44	1475	204	1.93
18	α -guaiane	50.33	1490	204	2.13
19	valencene	51.29	1506	204	0.31
20	cadina-1,4-diene	51.91	1516	204	0.55
21	eudesma-3,7(11)-diene	52.95	1534	204	0.10
22	cadina-3,9-diene	53.39	1542	204	1.60
23	germacrene B	54.44	1560	204	0.15
24	caryophyllene oxide	55.11	1572	220	24.02
25	6-epi-cubenol	56.60	1600	222	3.31
26	1,10-di-epi-cubenol	57.28	1610	222	0.16
27	epi- α -cadinol	59.26	1646	222	1.92
28	guai-1(10)-en-11-ol	59.96	1659	222	0.57
29	α -cadinol	60.19	1663	222	0.86
30	α -bisabolene oxide	61.08	1680	238	0.40
31	longipinene epoxide	63.90	1733	220	0.30
32	phytol	82.11	2110	297	0.62
Alkenes					25.87
Monoterpenes					14.2
Sesquiterpenes					20.23
Oxygenated compounds					33.11
Total					93.41

RT: Retention Time, RI: Retention Indice, m/z: Ratio Mass-Charge, % percentage

Table 2: Chemical composition of *E. praetermissa* aerial parts EO obtained by steam distillation

N°	Compounds	RT	RI	m/z	% Total
1	α -pinene	12.61	924	136	1.30
2	β -pinene	15.38	967	136	0.64
3	D-limonene	19.10	1023	136	1.4
4	1-undecene	23.87	1089	154	23.66
5	(+)-cycloisosativene	42.16	1358	204	4.72
6	β -elemene	43.95	1386	204	0.58
7	caryophyllene	45.43	1409	204	9.06
8	humulene	47.51	1443	204	1.31
9	germacrene D	49.59	1477	204	1.11
10	valencene	50.34	1490	204	3.53
11	cadina-1,4-diene	51.87	1516	204	0.30
12	cadina-3,9-diene	53.39	1542	204	1.02
13	caryophyllene oxide	55.09	1572	222	27.74
14	3, 4, 4-trimethyl-2-cyclohexen-1-one	56.60	1598	138	2.33
15	epi- α -Cadinol	59.26	1646	222	0.70
16	germacra-4(15), 5, 10(14)-trien-1 α -ol	61.08	1679	220	0.63
17	6, 10, 14-trimethylpentadecan-2-one	69.38	1839	268	7.63
18	phytol	82.06	2108	297	10.82
Alkenes					23.66
Monoterpenes					3.34
Sesquiterpenes					21.63
Oxygenated compounds					49.63
Total					98.26

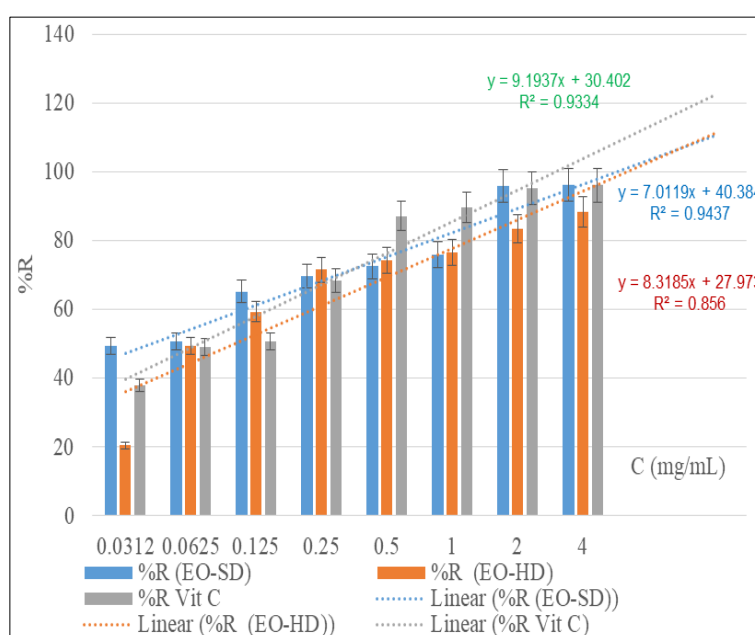
RT: Retention Time, RI: Retention Indice, m/z: Ratio Mass-Charge,%; percentage

This fraction is dominated by oxygenated compounds (49.63%), followed by alkenes (23.66%), sesquiterpenes (21.63%) and monoterpenes (3.34%). The main compounds identified are caryophyllene oxide (27.74%), 1-undecene (23.66%), phytol (10.82%) and caryophyllene (9.06%). Other minor, but notable, constituents include (+)-cycloisosativene (4.72%), valencene (3.53%), and the ketone 6, 10, 14-trimethylpentan-2-one (7.63%). Compared with the results of Rajesh ^[24], a slight difference in the chemical composition is observed. Indeed, our study reveals a higher content of oxygenated compounds and alkenes. This variation could reflect chemical diversity among different species of the genus *Emilia*, influenced by extraction methods or environmental conditions.

3.3 Antioxidant Potential of EOs

E. praetermissa essential oils (EOs) exhibit a better

antioxidant profile, as revealed by a comparative analysis of their DPPH-reducing power (Figure 1). The EO obtained by steam distillation (EO-EV) was found to be the most effective, with a CR₅₀ of 0.014±0.01 mg/mL, even surpassing that of vitamin C (CR₅₀ = 0.021±0.01 mg/mL) and that of the EO obtained by hydrodistillation (EO-HD, CR₅₀ = 0.026±0.01 mg/mL). According to Benov ^[25], EOs rich in oxygenated compounds generally possess higher antioxidant activity than those dominated by terpene hydrocarbons. This observation corroborates our results, since HE-EV, which has the highest proportion of oxygenated compounds (49.63%), also displays the best antioxidant efficacy. Similarly, HE-HD, although less effective, contains a significant proportion of these compounds (33.11%), which explains its notable antioxidant potential. Thus, the high content of oxygenated derivatives in these essential oils could be the origin of their remarkable antioxidant efficacy.



EO-SD: Essential Oil from Steam Distillation; EO-HD: Essential Oil from Hydro distillation

Fig 1: Comparative diagram of reduction percentage

3.4 Antimicrobial Activities of Essential Oils

The antimicrobial activity of the essential oil obtained by hydrodistillation of the aerial parts of *E. praetermissa* was evaluated by measuring the diameter of the microbial inhibition zone. Following the criteria of Berghe and Vlietinck ^[22], this EO showed variable efficacy: It showed no activity against *S. typhimurium*, *E. coli* and *P. aeruginosa* (00 mm inhibition diameter for each), but was moderately effective against *K. pneumoniae* (15 mm), *B. subtilis* (09 mm), *S. aureus* (12 mm), *S. epidermidis* (14 mm), *C. albicans* (12 mm), *C. tropicalis* (12 mm) and *C. glabrata* (11 mm). A bacteriostatic effect was also noted, particularly pronounced against *S. epidermidis* and *K. pneumoniae*. These results are consistent with previous research, including that of Afolayan *et al.* ^[11], who demonstrated the antimicrobial activity of solvent extracts obtained from *E. praetermissa* leaves. The observed minimum inhibitory concentrations (MIC) ranged from 3.125 mg/mL to 12.5 mg/mL, demonstrating comparable efficacy to commercially available antibiotics. It is important to note that, although all three extracts showed a weak effect against *Candida albicans* (inhibition zones of 3.33 mm to 7.33 mm), the essential oil (EO) of *E. praetermissa* demonstrated superior activity for this strain, with an inhibition zone of 12 mm. However, the EO inhibited *Staphylococcus aureus* and *Escherichia coli* less pronouncedly than the solvent extracts. These combined observations reinforce the idea that *E. praetermissa* has significant antimicrobial potential, making this plant a promising source for the discovery and development of new

bioactive compounds for therapeutic use.

3.5 Comparison of the antibacterial activity of *E. praetermissa* essential oils from the two extraction methods against *S. epidermidis* and *K. pneumoniae*

Among the bacterial strains tested, *Staphylococcus epidermidis* and *Klebsiella pneumoniae* emerged as the most sensitive during preliminary screening, justifying their selection for further comparative analysis. Inhibition tests revealed significant differences between the two extraction methods (Table 3). The essential oil obtained by steam distillation and that produced by hydrodistillation induced distinct inhibition zones, suggesting variation in their antibacterial efficacy. According to Kalembe and Kunicka ^[26], the antimicrobial activity of an essential oil is closely linked to its chemical composition. Although both EO share the same major compounds (caryophyllene oxide, 1-undecene, caryophyllene and phytol), their proportions differ, which could explain the variations observed in their inhibitory power. The results indicate that HE-HD exhibits slightly higher activity against *S. epidermidis* and *K. pneumoniae*, which could be explained either by a higher concentration of the active compounds responsible for the antibacterial effect, or by a synergy between several molecules, reinforcing the overall antimicrobial action. This study thus highlights that the extraction method influences not only the chemical composition of EOs, but also their biological efficacy, opening up perspectives for targeted applications depending on the bacterial strains to be combated.

Table 3: EO-HD and EO-SD inhibition zones against *S. epidermidis* and *K. pneumoneae*

Strains	EO-SD di (mm)	EO-HD di (mm)	Oxytetracycline (Reference) di (mm)
<i>K. pneumoneae</i>	13	15	30
<i>S. épidermidis</i>	14	15	30

4 Conclusion

This study demonstrated that the essential oil obtained from the aerial parts of *E. praetermissa*, characterized by a high concentration of oxygenated compounds, possesses significant antioxidant and antimicrobial activities. These properties scientifically support its widespread use in traditional medicine. Given these results, *E. praetermissa* represents a promising source for the isolation and identification of bioactive compounds for therapeutic or industrial purposes. However, it is crucial to note that the extraction method significantly influences the characteristics of the EO. Notable variations were observed between hydrodistillation and steam distillation, particularly in terms of color, yield, chemical composition, and biological activities. Differences in chemical composition have been reported, which directly translates into modulations in antioxidant and antimicrobial activities. It is therefore imperative to consider that each extraction method could be specifically adapted or optimized for a particular use, depending on the chemical profile and biological activities desired for the *E. praetermissa* EO.

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6. Declaration

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- **Ethical approval:** Not applicable.
- **Informed consent:** Not applicable

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