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Research Article

A Comparative Study on the Antioxidant and Insecticidal Activities of *Eucalyptus camaldulensis* Essential Oil and Hydrosol

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Abstract

The study investigates the hydrosol of *Eucalyptus camaldulensis* to assess its value and potential applications within the essential oil (EO) industry. Following steam distillation of *E. camaldulensis* leaves to extract essential oil, the resulting hydrosol underwent liquid-liquid extraction to yield a secondary essential oil (SEO). Comparative chemical analysis was performed using GC/MS. Comparative chemical analysis of the SEO and EO revealed distinct compositions, with 24 compounds identified in the SEO compared to 41 in the EO. Oxygenated compounds predominated in the SEO while non-oxygenated compounds were the most abundant in the EO (63.61%). Eucalyptol emerged as the primary constituent in both, with higher concentrations observed in the SEO (60.41%) than in the EO (28.53%). Additionally, antioxidant activity assessed using the DPPH assay demonstrated significant radical reduction in the SEO compared to the EO. In insecticidal tests targeting Callosobruchus maculatus, the SEO exhibited pronounced toxicity, with an IC_{50} value lower than that of the EO. This study showed the abundance of oxygenated compounds in *E. camaldulensis* hydrosol compared to pure essential oil, suggesting its potential as an antioxidant agent and for integrated pest management in agriculture. The research highlights the value of hydrosols in the EO industry and their potential applications in natural product formulations and pest control strategies.

Keywords

Floral Water, Eucalyptol, Antioxidant, Aromatherapy, Insecticide, Gas Chromatography

1. Introduction

Essential oils (EOs) derived from aromatic plants have gained significant popularity in recent years due to their natural and medicinal properties. With a global production exceeding 150,000 tons in 2017, valued at approximately 6 billion US dollars, the EO market has experienced remarkable growth [1]. These volatile compounds have been used for centuries in perfumes, food and beverages, as well as for their therapeutic effects on the body and mind [2-4]. EOs exhibit various modes of action, including repellent, inhibitory, and growth-reducing properties, making them valuable in antiparasitic, bactericidal,

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fungicidal, antiviral, and insecticidal applications [1].

Hydrosols, also known as hydrolats or aromatic waters, are produced during the steam distillation process used to extract essential oils. While the EO is collected as the upper phase after condensation, the remaining water contains dissolved constituents from the oil, providing unique organoleptic and biological properties [5]. Unfortunately, hydrosols are often discarded, resulting in the loss of valuable aromatic compounds. Various methods have been explored to recover these dissolved oils, leading to the production of secondary EOs [5].

Although hydrosols are used in cosmetics, food, and aromatherapy, their potential remains largely unknown. This is particularly true for *E. camaldulensis*, a widely cultivated species in West Africa known for its EO production. While the EO is highly valued, the hydrosol is often overlooked. To the best of our knowledge, no scientific studies have focused on hydrosols of *E. camaldulensis* from West Africa. Therefore, the main objective of this study was to enhance the value of *E. camaldulensis* hydrosols. Specifically, we aimed to analyse the chemical composition of the hydrosol compared to its corresponding EO; evaluate their antioxidant potential and assess their insecticidal activity against cowpea bruchids *Callosobruchus maculatus*. By shedding light on the potential benefits of hydrosols, this research aims to contribute to their utilization in various fields.

2. Materials and Methods

2.1. Plant Material

Leaves and small stems of *E. camaldulensis* were collected from the University of Lomé, Togo, located at coordinates Latitude 6,17369 °N and Longitude 1,21446 °E. The collection was made in August 2021 and the plant material was identified and registered as TOGO15878 in the herbarium of the Department of Botany and Ecology at the University of Lomé.

2.2. *Callosobruchus Maculatus* Population

To keep insect population for insecticidal assays, cowpea seeds (*Vigna unguiculata*) were obtained from a local market and subjected to a freezing process for sterilisation. After a period of 15 days, the seeds were taken out and allowed to acclimatize in the open air for approximately one hour. Petri dishes with a diameter of 14 cm, containing approximately 100 g of these seeds each were prepared. Twenty pairs of *C. maculatus* were added to each dish. The dishes were then incubated under controlled laboratory conditions at a temperature of 28 ± 2 ℃ and a humidity of 80 ± 10%. After 48 hours, the insects laid eggs on the seeds, which were subsequently removed from the dishes. The seeds were monitored until a homogeneous generation of insect adults was obtained, typically around 30-32 days after egg laying. The adults were used for toxicity tests following the method described by Dick and Credland (1984) [6].

2.3. Essential Oil Extraction

The EO and its corresponding hydrosol were obtained through the process of steam distillation. A total of 7.34 kg of leaves and small stems were used as biomass for the distillation. Prior to distillation, the biomass was dried for 72 hours under laboratory conditions at a temperature of 26 ± 2 ℃, while being protected from light. The plant materials were submitted for 4 h to hydrodistillation using a Clevenger-type apparatus using the method reported in *European Pharmacopoeia*. The EOs were obtained on the top of hydrosols and dried on anhydrous magnesium sulfate before stored in amber vials in the refrigerator at 4 ℃.

2.4. Secondary Essential Oil Extraction

The secondary essential oils (SEO) were obtained from the previous hydrosols through evaporation after liquid-liquid extraction using hexane. In a separating funnel, 40 mL of hexane was combined with 200 mL of hydrosol and mix thoroughly to extract volatile compounds. For this experiment, a total of 5 L of *E. camaldulensis* hydrosol were used. After the liquid-liquid extraction, the SEOs were collected in vials and stored in a refrigerator at a temperature of 4 ℃, while being protected from light. These SEO samples were kept under appropriate storage conditions until they were used for further analysis.

2.5. Yield of Primary Essential Oils

Essential oil yield Y was defined as the ratio between the mass of the extracted essential oil and the mass of the dry plant material used. It was expressed as a percentage and calculated according to the following formula:

$$Y(\%) = (W_{EO}/W_b)\ X100$$

With W_{EO} the essential oil weight and W_b the biomass weight.

2.6. Mass Concentrations of Hydrosols in Secondary Essential Oils

The secondary essential oil concentration C was defined as the ratio of the mass of the secondary essential oil obtained after liquid-liquid extraction and the volume of hydrosol used. It was expressed in (g/L).

$$C(g/L) = (\text{Secondary EO mass})/(\text{Volume of hydrosol})$$

2.7. Gas Chromatography-Mass Spectrometry Analysis

The chemical composition of the essential oil and hydrosol was determined using gas chromatography coupled with mass spectrometry (GC-MS). The analysis was conducted using a

Thermo Scientific Trace 1300 gas chromatograph, coupled to a Thermo Scientific ISQ QD selective mass detector and a 7683B Injector Autosampler series automatic sample injector. The injection was performed in splitless mode. The column used was made of 5% polysiloxane phenyl, measuring 30 meters in length, with an internal diameter of 0.25 mm and a film thickness of 0.25 µm. The injector temperature was maintained at 250°C. The oven temperature was programmed to increase from 50 to 230 ℃ at a rate of 5 ℃ per minute and held isothermal for 2 minutes, resulting in a total run time of 38 minutes. The essential oil samples were diluted 10,000 times with hexane. Helium was used as the carrier gas with a flow rate of 1 mL/min, and 1 µL of the sample was injected. The mass spectrometer operated in the mass range of 40-350 m/z with an ionization energy of 70 eV, and the total ion current (TIC) chromatograms were recorded. The obtained data was processed using XCALIBUR software. The identification of essential oil components was performed by comparing their retention times and mass spectra with those of authentic standards, if available. Kovats' indices were utilized for compound characterization.

2.8. Antioxidant Activity

The antioxidant activity of the essential oils (EO) was evaluated using the diphenylpicrylhydrazyle (DPPH) method. The protocol followed in this study was based on the method described by Agbodan et *al*., with slight modifications [7]. The DPPH reagent, known for its instability, was freshly prepared daily at a concentration of 98-100 µmol/L. To ensure the reliability of the experiment, the absorbance of the solution was measured using a spectrophotometer (DMS 300 UV-visible spectrophotometer) at a wavelength of 517 nm after each preparation. An absorbance reading in the range of 0.9-1.0 confirmed the reagent's stability. The tested solutions were titrated in the range of 0-5 µg/mL. Quercetin, a standard antioxidant, was used as the positive control, and its absorbance was measured under the same conditions as the test samples. Three replicates were performed for each concentration, and the mean optical densities were calculated. The percentage of inhibition was determined based on the optical densities and the concentrations of the essential oils using the following formula:

$$\text{Percentage of inhibition} = \frac{\text{Absorbance (negative control))} - \text{Absorbance (EO)}}{\text{Absorbance (negative control)}} * 100$$

The IC_{50} colour intensities were determined from the equations of the corresponding regression curves. IC_{50} represents the amount of product needed to reduce the staining of the reagent solution (DPPH) by half, or 50%.

2.9. Insecticidal Activity of Essential Oils

To assess the insecticidal activity of the hydrosol and essential oil, glass jars with a capacity of 1L were used. Each jar contained 20 couples of *C. maculatus* adults obtained from the breeding program. A filter paper disc with a diameter of 2.1 cm was placed inside each jar. For the test, different volumes of essential oil (3, 5, 8, and 12 µL) were applied onto the filter paper discs, allowing the aroma of the essential oil to permeate the medium. Four replicates were conducted for each volume of essential oil. In these experiments, an insect was considered dead when its antennae and legs showed no movement after a slight disturbance using pliers. The control group of insects was kept under the same environmental conditions, with the only difference being that the filter paper disc in this case was not soaked with essential oil. The concentrations of the essential oil are expressed in terms of the volume of the jar and the amount of essential oil deposited on the filter paper disc (µL/L).

2.10. Statistical Analysis

Statistical analysis was performed using GraphPad 8.0.2 and Minitab Statistical Software. The means of the IC_{50} values obtained from the antioxidant tests using $DPPH^{\cdot}$ and the IC_{50} values from the toxicity tests were reported along with the corresponding Standard Error of the Mean (SEM). Significance tests provided standard deviations and P-values at the 5% level.

3. Results

3.1. Extraction Yields and Concentrations of Hydrosols in Secondary Essential Oils

From the dried plant materials, the EO yield was 0.45% and the hydrosol concentration in the hydrolates was 0.38 g/L as calculated from the SEO weights.

3.2. Gas Chromatography Coupled with Mass Spectrometry Analysis

After GC-MS analysis, the chemical compositions of both essential oil types were determined. Table 1 shows the components identified with their relative percentage compositions.

Table 1. *Chemical composition and percentages of EO and SEO constituents of E. camaldulensis.*

N °	RI	RI r	Components	EO%	SEO%
			Monoterpenes	57	0
1	900	937	α-Pinene	20.14	-
2	963	979	β-Pinene	12.89	-
3	989	991	β-Myrcene	0.41	-
4	1003	1005	α-Phellandrene	0.11	-
5	1019	1017	α-Terpinene	0.18	-
6	1070	1060	γ-Terpinene	23.59	-
7	1100	1088	Terpinolene	1.25	-
			Oxygenated monoterpenes	33.44	98.92
1	1036	1032	Eucalyptol	28.53	60.41
2	1085	1102	Cis-p-Mentha-2,8-dien-1-ol	0.13	0.13
3	1116	1115	Linalol	-	0.14
4	1124	1126	p-menth-2-en-1-ol	0.73	-
5	1125	1135	Fenchol	-	2.49
6	1147	1144	trans-Verbenol	0.76	-
7	1147	1142	cis-Verbenol	-	4.90
8	1154	1153	β-Terpineol	0.09	-
9	1155	1155	Camphene hydrate	-	0.34
10	1144	1141	(E)-Myroxide	-	1.34
11	1170	1167	Borneol	0.61	-
12	1150	1157	Iso borneol	-	3.79
13	1179	1177	Terpineol	1.72	-
14	1185	1189	α-Terpineol	-	18.97
15	1190	1190	α-Thujenal	-	0.60
16	1195	1195	Myrtenol	0.36	-
17	1192	1193	Myrtenal	-	2.14
18	1214	1219	Carveol	0.05	0.43
20	1220	1220	β-Cyclocitral	0.03	-
21	1221	1217	(E)-Carveol	-	0.28
22	1227	1226	Dihydrocarveol	0.03	-
23	1231	1229	cis-Carveol	0.05	-
24	1232	1228	D-Verbenone	-	0.38
25	1234	1231	cis-Tagetenone	0.05	-
26	1248	1240	β-Citral	-	0.43
27	1258	1255	Carvenone	0.07	-
28	1259	1255	Carvenone	-	0.44
29	1279	1276	Citral	0.03	0.45
30	1301	1299	4,5-Dimethyl-2-Carvacrol	0.12	-

N °	RI	RI r	Components	EO%	SEO%
32	1302	1299	Carvacrol	-	0.65
32	1310	1305	6-Ethyl-3,4-dimethylphenol	-	0.44
33	1314	1314	Car-3-en-5-one	0.08	-
34	1362	1305	p-Eugenol	-	0.17
			Sesquiterpenes	2.75	0
1	1371	1372	1H-Indene, 2, 3,3, 4-tetrahydro-3,3a,6-trimethyl-1-(1-methylethyl)-	0.04	-
2	1434	1432	β-Gurjunene	1.59	-
3	1439	1439	4-Isopropyl-1,6-α-Guaiene	0.06	-
4	1458	1459	Dimethylenedecahydronaphthalene	0.36	-
5	1471	1473	γ-Gurjunene	0.03	-
6	1477	1477	γ-Muurolene	0.03	-
7	1484	1486	β-Selinene	0.06	-
8	1495	1494	α-Selinene	0.34	-
9	1514	1513	γ-Cadinene	0.02	-
10	1524	1524	δ-Cadinene	0.06	-
11	1556	1557	Germacrene B	0.16	-
			Oxygenated sesquiterpenes	0.28	0.53
1	1562	1565	Ledol	0.07	-
2	1584	1585	Epiglobulol	0.13	-
3	1593	1591	Himbaccol	0.08	-
4	1396	1394	(Z)-Jasmone	-	0.15
5	1578	1381	(E)-Sesquisabinene hydrate	-	0.38
			Total oxygenated compounds	35.57	99.88
			Total non oxygenated compounds	63.61	0

RI: Retention Index; RI r: Retention Index reference

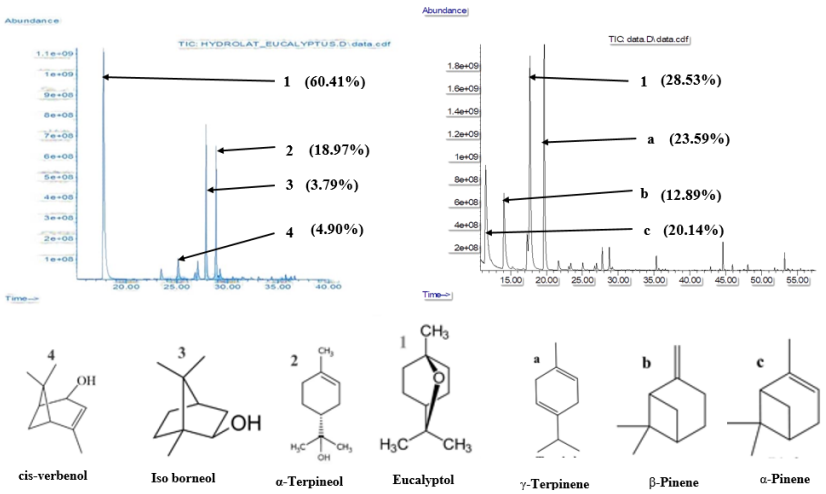

Figure 1. *Chromatograms of hydrosols (blue) and essential oil (black) of E. camaldulensis and structures of the majority compounds of these essential oils.*

Figure 1 describes chromatograms and main compounds detected in the EO and SEO of *E. camaldulensis*.

3.3. Antioxidant Activity Evaluation

Table 2 presents the equations of the calibration curves of the EO, SEO and quercetin samples and the different IC_{50} determined using these equations. The p-values between the IC_{50} of SEO and EO was 0.014. The hydrosols are then more antioxidant than the main essential oils of *E. camaldulensis*.

Table 2. *DPPH antioxidant activities of E. camaldulensis EO and SEO.*

Extract	Regression line equation	IC_{50} (µg/mL)
EO	Y = 1,08130*X + 8,02044	33,70 ±4,36
SEO	Y = 5,62549*X + 4,90413	8,07 ±0,16
Quercetin	Y = 17,2123*X + 8,54129	2,44 ±0,26

3.4. Insecticidal Activities of Essential Oil

The hydrosols and the essential oil of *E. camaldulensis* showed differential toxicity to *C. maculatus* after 24 h of exposure with IC_{50} values of 4.31 ± 1.35 and 7.67 ± 3.07 µL/L air, respectively.

4. Discussion

The extraction yields of *E. camaldulensis* essential oil were determined to be 0.45%, that is slightly lower than the yield reported by Azzi et *al*., using hydrodistillation which was 0.58% [8]. That extraction is very lower than the yield reported by Tine et *al*., using also hydrodistillation, which was 4.35% [2]. The difference in extraction technique may account for this variation in yields. It is important to note that different extraction methods and equipment can lead to different yields. Additionally, soil typology and the plant's development environment can also influence the concentration of species in the plant [9, 10].

Eucalyptol is the main constituent identified in the extract. Eucalyptol was predominant in both, with higher concentrations in the SEO. Our result is similar to Tine et *al*., [2].

The hydrosol obtained during the extraction process was found to have a notable concentration of essential oil, specifically 0.378 g/L for *E. camaldulensis*. This indicates that the hydrosol is a valuable source of the secondary essential oil. However, it is important to consider that the concentration of essential oil in the hydrosol can be influenced by various factors, such as the polarity of the liquid-liquid extraction solvent and the specific technique used for hydrosol extraction. These variables can affect the efficiency of SEO extraction and subsequently impact the concentration of SEO present in the hydrosol. Therefore, it is essential to carefully select the appropriate extraction method and solvent polarity to optimize the yield and concentration of essential oil in the hydrosol.

In terms of insecticidal activity, both the EO and SEO of *E. camaldulensis* exhibited high toxicity against *C. maculatus*, even at low concentrations. Our results are consistent with the findings of Togola et *al*., who demonstrated the effectiveness of *E. camaldulensis* essential oil against insect pests of rice storage [11]. We observed a significant toxicity of these essential oils on *C. maculatus*, with respective IC_{50} values of 4.31 ± 1.35 µL/L air and 7.67 ± 3.07 µL/L air. Interestingly, the hydrosol exhibited even greater insecticidal activity compared to the essential oil, likely due to its higher content of oxygen compounds. Oxygenated terpene compounds have been shown to possess greater biological activities than non-oxygenated terpenes [12]. Thus, the hydrosol of *E. camaldulensis* holds promise for post-harvest protection. To the best of our knowledge, this study is the first to evaluate the efficacy of the hydrosols of this plant against cowpea bruchids. The insecticidal activity of *E. camaldulensis* essential oil is likely attributed to the presence of eucalyptol. Notably, the hydrosol contained more than double the amount of eucalyptol compared to the essential oil (60.41% and 28.53%, respectively), which explains the hydrosol superior insecticidal activity. Several studies have demonstrated the antimicrobial properties of eucalyptol-rich essential oils from eucalyptus species against a wide range of micro-organisms [13-15].

However, it is important to consider that all components of the essential oil may interact and modulate each other activities, including the minor constituents [10].

Furthermore, our study revealed that the hydrosol exhibited a higher anti-radical power compared to its essential oil, as indicated by the inhibitory concentrations IC_{50} of 8.074 ± 0.157 and 33.704 ±4.363, respectively. The GC-MS analysis results supported these findings, showing that the hydrosol contained a higher concentration of hydrophilic oxygen molecules and a lower presence of lipophilic compounds such as terpene hydrocarbons. This observation aligns with the findings of Piochon et *al*., [16]. The notable difference in chemical compositions between hydrosols and essential oils may account for the superior anti-radical activity of the hydrosol.

Further investigations on antioxidant activity and extraction by other solvents could be clarify activities of *E. camaldulensis*.

5. Conclusion

The conclusion of this study on *E. camaldulensis* has shown the considerable potential of essential oils hydrosols, usually neglected, in containing potent bioactive compounds. *E. camaldulensis* hydrosols revealed more bioactive oxygen-

ated compounds in SEO than EO with Eucalyptol the main compound identified. These hydrosols demonstrated promising antioxidant and insecticidal properties, indicating their potential application in agricultural and natural product development. Future research focusing on hydrosols from different plant sources and their synergistic effects with essential oils could show novel bioactive compounds and further enhance their biological activities. Exploring these avenues holds promise for discovering sustainable solutions across diverse fields.

Abbreviations

EO: Essentiel Oil
SEO: Secondary Essentiel Oil
DPPH: Diphenylpicrylhydrazyle

Author Contributions

Affo Dermane: Conceptualization, Resources, Data curation, Formal Analysis, Supervision, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing

Kodjo Eloh: Conceptualization, Formal Analysis, Supervision, Funding acquisition, Methodology, Writing – original draft, Project administration, Writing – review & editing

Oudjaniyobi Simalou: Formal Analysis, Supervision, Visualization, Writing - review & editing

Balabapat é Assiki: Formal Analysis, Investigation, Writing - original draft, Methodology, Visualization, Writing - review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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