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PARTICLE SIZE EFFECTS ON ANTIOXIDANT POTENTIAL AND LARVICIDAL EFFICACY OF ESSENTIAL OIL FROM *Lippia chevalieri* (Mold) LEAVES ON *Anopheles gambiae* s.l. (Diptera: Culicidae)

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Abstract: A new approach to enhancing plant activities is encouraged. Fresh leaves of *Lippia chevalieri* were dried, ground and fractionated into granulometric classes (≤ 212 µm; 212 - 425 µm; ≥ 425 µm) by sieving. The essential oils (EOs) were analyzed by gas chromatography (GC) coupled with mass spectrometry (MS). Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) tests. Larvicidal efficacy was tested on *Anopheles gambiae* s.l. under laboratory conditions. Particle size had a significant influence ($p < 0.05$) on the phytocompounds, antioxidant and larvicidal properties of the EOs. Comparatively, the number of phytochemicals decreases as we move towards fine particles. The ≤ 212 µm EO fraction exhibited higher DPPH (IC_{50} = 78.2 ± 2.24 µg/mL) and FRAP (48.72 ± 0.028 mgAAE/gEO) activities than did ascorbic acid. Fresh leaves, dry leaves, unsieved powder and large particles (≥ 425 µm) induced significant ($P < 0.05$) concentration-dependent larvicidal activity, with LC_{50s} of 1,93, 1.55, 1.68 and 2.11 mg/mL, respectively. Grinding followed by sieving results in the accumulation of the majority of antioxidant compounds in the EO of fine particles. Drying and grinding increases the larvicidal efficacy of the plant, while fractioning into fine particles progressively reduces the efficacy as a function of particle size.

Keywords: *Lippia chevalieri*, Particle size fractionation, Essential oil, Antioxidant activity, Larvicidal activity, *Anopheles gambiae*.

1. INTRODUCTION

Medicinal and aromatic herbs have been used by many people around the world to control a variety of diseases and wounds since ancient times, particularly in low and middle-income nations where people face difficulties accessing modern medications (**Salamatullah, 2022**). Plants are known to be a natural resource widely exploited by humans to fight against pathologies of oxidative and vector origin (**Salamatullah, 2022**). This is why the last two decades have witnessed a proliferation of research and methods for extracting antioxidants and larviciding molecules from plants. Furthermore, the production of plant powders to concentrate and optimize the extraction of biomolecules as well as their functional properties is an area of growing interest **(Zaiter *et al.*, 2016; Deli *et al.*, 2019)**. Recent studies have shown that particle size has a significant influence on the antioxidant and hepatoprotective potential of EOs **(Noumi *et al.*, 2022)**. *Lippia chevalieri* Mold is a Sudanese Guinean plant of the Verbenaceae family. This plant is used in traditional medicine to treat respiratory diseases, liver pathologies, buccoanal and digestive candidiasis, anemia, malaria, painful periods, insomnia, nervousness and fever **(Bangou *et al.*, 2012)**; it has also demonstrated larvicidal effects on the mosquito species *Anopheles gambiae* s.l., *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles funestus* **(Wangrawa *et al.*, 2022a, 2022b).** The objective of this study is to assess the effect of particle size fractionation by sieving on the concentration of phytochemicals, antioxidant properties and larvicidal activities of EOs from *L. chevalieri* leaves fractions against *Anopheles gambiae*.

2. MATERIALS AND METHODS

2.1. Plant Materials

2.1.1 Plant harvesting and processing

This study and collection of plant data were carried out with the academic authorization of the head of the Department of Animal Biology and Physiology of the Faculty of Science at the University of Yaounde 1, Cameroun. The study complied with all relevant regulations and guidelines.

Fresh leaves of *L. chevalieri* were collected from the Wack locality (Ngaoundéré, Cameroon: latitude 7°40'43''; longitude 13°33'20'') in November 2021. Identification was performed by the head of the National Herbarium of Cameroon in comparison with the material of R. Letouzey n° 7349 from the herbarium collection N°9057 SRFT/Cam of the National Herbarium of Cameroon. After washing and cleaning, the leaves were dried at room temperature, which varied from 14°C to 38°C, for seven days.

2.1.2. Plant grinding and particle size analysis

Dried leaves of *L. chevalierie* were ground in a robot blender mill. The analytical method used by **Nguimbou *et al.* (2020)** was applied for the analysis of the particle size distribution of the unsieved powder. Measurements were carried out using a laser from a Mastersizer 3000 (Malvern Instruments, Orsay, France) supplied with an Aero S wet dispersion unit. The chosen size estimator, was the particle size in volume and classical granulometric parameters were determined: D10, D50 and D90 (where Dx means that x% of the volume of particles has a diameter inferior to Dx). The span, a common parameter related to the width of the particle size distribution, was calculated as thus:

$$Span = \frac{\mathrm{D90} - \mathrm{D10}}{\mathrm{D50}}$$

2.1.3. Powder sieving

Plant powder was separated into granulometric classes using a series of two sieves of various apertures (212 µm and 425 µm) selected on the basis of particle size analysis. The powder was sieved according to the procedure used by **Noumi *et al.* (2021)**. The following powder fractions were obtained: ≤ 212 µm, 212-425 µm and ≥ 425 µm with a moisture content of approximately 10-12%. Unsieved powder was used as a control. The powder samples were stored at 10°C in polyethylene bags and placed at room temperature (25°C ± 2°C) until they were used.

2.1.4. Essential Oil Extraction

EO was extracted from each sample by hydro distillation performed via a setup recognize as the Clevenger apparatus or simple steam distillation for 5 hours. The EO obtained was subsequently introduced into dark bottles and stored at 4°C protected from light. The extraction yield was expressed as the mass of distillate (m) per mass of dry leaves (Ms) and calculated as follows:

$$Yield\ (\%) = \frac{\mathrm{m}}{\mathrm{Ms}}\ \mathrm{X}\ 100$$

2.1.5. Analytical conditions

The analysis of the EO was carried out using a Varian CP-3380 type chromatograph equipped with a flame ionization detector and a capillary column (30 m × 0.25 mm) with a stationary apolar phase of the methylsilicone type (DB5, film thickness 0.25 µm). The retention indices of the constituents were determined relative to the retention times of a series of n-alkanes. The coupling gas chromatography–mass spectrometry was carried out using an apparatus (Hewlett-Packard HP 5970 A) equipped with an apolar capillary column (30 m × 0.25 mm) in fused silica of type HP-1 (film thickness 0.25 µm) and a quadrupole type detector (ionization energy 70 eV). The components were identified on the basis of their retention indices and mass spectra by comparison with data from the National Institute of Standards and Technology (NIST) **(Adama, 2007)**. The percentages of the compounds were calculated from the GC peak areas using the normalization method.

2.2. Mosquito strain

Anopheles gambiae eggs were obtained from the Organization for Coordinating the fight against Epidemics in Central Africa (OCEAC) and reared in the insectarium of the Zoology Laboratory of the Higher Teacher's Training College of Yaoundé under ambient conditions (27 ± 2°C; 74 ± 4% R.H.) according to the standard World Health Organization (WHO) protocol **(WHO, 2005)** to obtain third- and fourth-instar larvae, which were used for larvicidal tests**.**

2.3. Antioxidant activity evaluation of the EOs

2.3.1. DPPH Radical Scavenging Capacity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) anti-free radical activity of the EO samples was evaluated in accordance with the methods of **Zhang and Hamauzu (2004)** with slights modifications. Then, 0.5 mL of the methanolic solution of each EO at different concentrations was added to 1 mL of the DPPH solution (0.025 g/L). In parallel, a negative control was prepared by mixing 0.5 mL of methanol with 1 mL of the methanolic solution of DPPH. The optical density was recorded at 517 nm using the methanolic solution for the blank tube and ascorbic acid as a standard after 60 min of incubation in the dark and at laboratory temperature (25°C ± 2°C). The percentage of free radical scavenging effect was calculated as follows:

$$\text{DPPH scavenging (\%)} = \frac{\text{DO control} - \text{DO sample}}{\text{DO control}} \text{X } 100$$

2.3.2. Ferric reducing antioxidant power (FRAP) assay

Fresh FRAP reagent was prepared according to the methods of **Zaouali, (2013)** by combining TPTZ solution (10 mM TPTZ in 40 mM HCl), ferric solution (20 mM, $FeCl_3$, $6H_2O$) and acetate buffer (300 mM, pH 3.6) at a ratio of 1:1:10 (v/v). The reaction was then carried out by mixing 900 µl of FRAP solution with 90 µl of distilled water and 30 µl of the diluted samples. The mixture was incubated at 37°C for 30 min, after which the absorbance was read at 593 nm. The ferric reducing power was determined from the calibration curve drawn from different concentrations of iron sulfate solution ($FeSO_4$, $6H_2O$). The results are expressed in mmol Fe^{2+} per gram of essential oil (mgAAEFe^{2+}/g EO).

2.4. Larvicidal efficacy of Lippia chevalieri fractions as essential oils against Anopheles gambiae.

The larvicidal activities of *Lippia chevalieri* Fresh Leaves, Dry Leaves, Unsieved Powder, Large Particles ($P \geq 425$ µm), Median Particles (212 – 425 µm) and Fine Paricles ($P \leq 212$ µm) essential oils were evaluated on a blend of 3rd and 4th instar larvae of *Anopheles gambiae* according to the WHO standard protocol **(WHO, 2005).** After the preliminary tests, a stock solution was prepared with a concentration of 5 mg/mL by dissolving 100 mg of each essential oil in 20 mL of ethanol in a glass flask. Test concentrations of 1, 2, 3 and 4 mg/mL were then prepared from the stock solution. Twenty-five, third- and fourth-instar larvae were transferred into 250 mL plastic cups containing spring water, to which 1 mL of test solution of different concentrations was added, and the volume was increased to 100 mL per cup. The negative control consisted of adding 1 mL of ethanol to 99 mL of spring water, while the positive control was a solution of Dichlorvos DDVP (1 mg/mL). Each concentration was tested four times, and larval mortality was recorded after 24 hours postexposure.

2.5. Statistical analysis

The statistical significance of differences between sample means was determined using analysis of variance (ANOVA) followed by Duncan's test at the 95% confidence level using Stata 15.1. Principal component analysis (PCA) was used to classify and correlate the factors that define powder fractions, phytochemical compounds and antioxidant activities realized by XL-STAT 2019. Probit analysis was performed to determine the lethal concentration that caused 50% (LC50) and 95% (LC95) mortality in the mosquito larvae.

3. RESULTS AND DISCUSSION

3.1. Particle size characteristics of the unsieved powder

Figure 1 shows the distribution curve of the unsieved powder of *L. chevalieri* obtained with the laser granulometer. The results showed that the powder had a homogeneous distribution. According to **Fournier *et al*. (2012)** powder is said to be homogeneous when it has the shape of a narrow bell. Table 1 shows the particle size characteristics of the unsieved powder of *L. chevalieri* leaves. We observed a volume of fine particles of approximately 50.09 ± 2.1 µm (D10), a median volume of particles of approximately 362.1 ± 5.62 µm (D50) and a volume of large particles of approximately 750.33 ± 9.67 µm (D90). The major volume corresponded to large particles, whereas fine particles were less common (10% of the total volume of the powder). The low span value of 2.048 reflects the low dispersion of the unsieved powder and confirmed that the grinding process was complete. Indeed, dispersion is said to be wide if its span is greater than three **(Zaiter *et al*., 2016).** The fact that the major volume corresponded to large particles can be explained by the expected non-sphericity of large particles, as ground fibers generally lead to large rod-shaped particles. Therefore, fine particles can stick to larger particles and thus, be retained by sieves with mesh sizes exceeding their diameter **(Deli *et al*., 2019).** Given that the median distribution of the mother powder was approximately 300 µm, the fractionation sieves were chosen as follows: a 212 µm sieve for studying particles below the median and a 425 µm sieve for studying particles above the median.

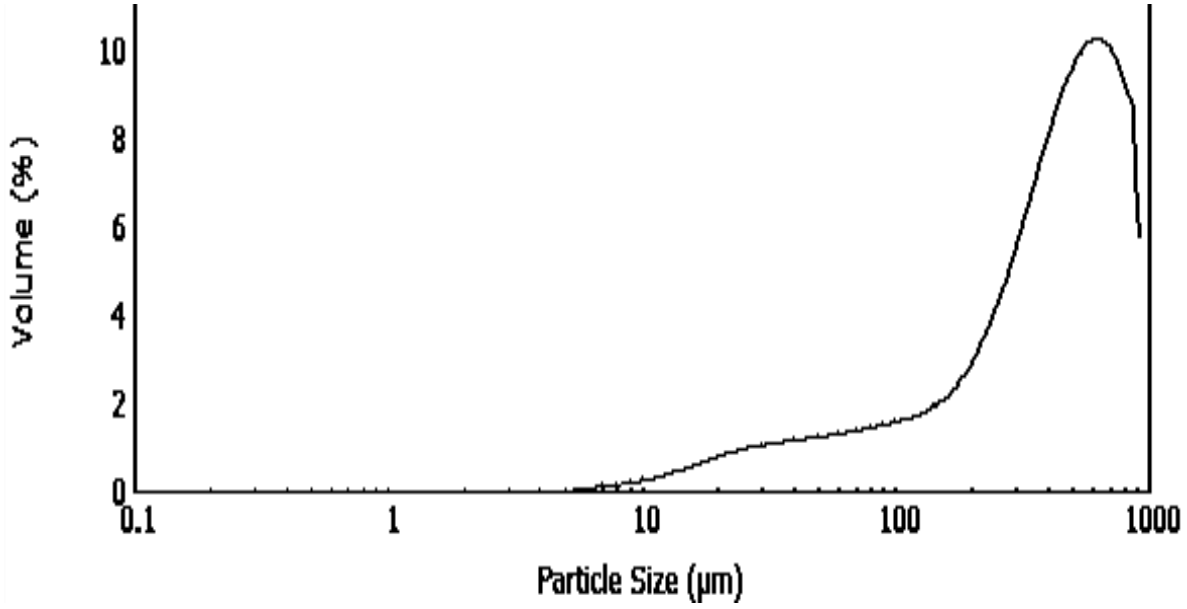


Figure 1: Distribution curve of unsieved powder of *L. chevalieri*.

Table 1. Particle size characteristics of the unsieved powder of *L. chevalieri*.

Characteristics (µm)	**Values**
D10	50,09 ± 2.1
D50	362,1 ± 5,62
D90	750,33 ± 9.67
Span	2,048 ± 0,024

3.2. Extractions yields and chemical compositions of Lippia chevaleri fractions.

3.2.1. Extraction Yields

Table 2 presents the extraction yields of the different samples. It is noted that there is a considerable increase in yield when the leaves are dried compared to when fresh leaves are used. This result can be explained by the fact that after drying, the mass of the leaves decreases. Consequently, more dry leaves are needed to obtain the desired total mass. This translates into an increase in dry matter and therefore an increase in yield. In addition, the dried leaves of *Lippia chevaleri* were ground before distillation. At this level, the yield decreases, and this result can be explained by the fact that the thermogenic grinding temperature of particles in the surrounding grinder can reach 90°C, which can induce the release of essential oils and volatile organic compounds with low molecular weights [15]. In these works, Zrira and Benjillali[16] reported a decrease in yield between leaves that were dried (1.42%) for 16 days and those that were crushed into fine powder (1.29%).

Table 2: Extraction yields of different samples.

Samples	Mass (g)	EO (g)	Yield (%)
≤ 212 µm	152	0.28	0.19 ± 0.1^{a}
212 – 425 µm	808	7.27	0.9 ± 0.1^{b}
≥ 425 µm	1039	15.59	1.5 ± 0.1^{c}
Unsieved powder (UP)	2000	120	6 ± 0.1^{d}
Dry leaves (DL)	2000	204	10.2 ± 0.1^{f}
Fresh leaves (FL)	2000	23	1.15 ± 0.1^{c}

The means ± standard in the Yield column followed by different letters were significantly different ($p < 0.05$) according to Duncan's multiple range test ($n = 3$).

3.2.2. EO chemical composition

Table 3 shows the analytical results for phytochemicals in EOs from all granulometric classes, as well as for the unsieved powder, fresh and dry leaves of *L. chevalieri*. Most of the constituents identified in the EOs of fresh leaves were monoterpenes (77.45%), with beta-cymene (33.84%), thymol (21.6%) and carvacrol acetate (11.64%) being the majority compounds. The EOs found in the dried leaves were 1,3,8-p-menthane (31.84%), gamma terpinene (20.16%), thymol (11.13%) and beta-myrcene (9.84%). After drying, we also noted the presence of new terpene compounds (alpha-thujene, alpha-terpinene, limonene, linalool, and geraniol) in the EOs of the dried leaves, which were absent in the EOs of the fresh leaves.

Table 3. Chemical composition (%) of EOs of the different granulometric classes, mother powder, fresh and dry leaves of *L. chevalieri*.

Compounds	Retention time (min)	Fresh leaves	Dry leaves	Unsieved powder	≥425 µm	212-425 µm	≤212 µm
alpha.-Thujene	9.492	---	4.29	---	---	---	---
beta.-Myrcene	13.165	---	**9.84**	---	**7.55**	---	---
alpha.-Terpinene	13.795	---	5.81	---	2.00	---	---
Limonene	14.354	---	1.54	---	1.38	---	---
gamma-Terpinene	15.765	6.45	**20.16**	---	**10.63**	2.15	---
o-Cymene	16.444	---	---	---	---	5.89	---
1,3,8-p-Menthatriene	16.477	---	**31.84**	---	**29.69**	---	---
beta-Cymene	16.519	**33.84**	---	---	---	---	---
cis-Sabinene hydrate	21.774	---	---	---	2.61	---	---
Linalool	23.678	---	1.15	---	1.87	---	---
(E)-p-Mentha-2-en-1-ol	24.416	---	---	---	0.10	---	---
4-Terpineol	25.459	3.87	1.98	4.44	4.41	---	---
Caryophyllene	25.636	5.83	5.77	**18.01**	**14.00**	**30.82**	---
beta.-Farnesene	26.682	5.29	2.22	**12.90**	**9.10**	**22.65**	**10.32**
Humulene	27.429	3.23	---	**10.30**	3.59	**7.72**	---
beta.-Bisabolene	28.369	---	---	---	---	2.74	---
beta-copaene	28.376	---	---	**27.09**	---	---	---
alpha-Farnesene	28.723	---	---	7.48	---	---	---
Geraniol	30.899	---	1.27	---	---	---	---
Thymol acetate	31.154	---	---	---	---	**9.00**	---
Carvacryl acetate	31.666	**11.64**	1.89	**8.01**	1.53	---	---
Caryophyllene oxide	34.677	**8.20**	1.11	**9.96**	**11.54**	**17.26**	**23.91**
Isothymol	38.141	---	---	---	---	0.85	---
Carvacrol	38.452	---	---	---	---	---	**65.77**
Thymol	38.464	**21.65**	**11.13**	---	---	0.92	---
Farnesyl acetate	39.356	---	---	1.81	---	---	---
monoterpenic hydrcarbons		**40.29**	**73.42**	---	**51.25**	**8.04**	---
Monoterpenic oxygenated		**37.16**	**17.42**	**12.45**	**10.52**	**10.77**	**65.77**
Sesquiterpenic hydrocarbons		**14.35**	**7.99**	**75.78**	**26.69**	**63.93**	**10.32**
Sesquiterpenic oxygenated		**8.10**	**1.11**	**11.77**	**11.54**	**17.26**	**23.91**
Terpenic hydrocarbons		**54.64**	**81.41**	**75.78**	**77.94**	**71.97**	**10.32**
Terpenic oxygenated		**45.26**	**18.5**	**24.22**	**22.06**	**28.03**	**89.68**
Total		**99.9**	**99.94**	**100**	**100**	**100**	**100**

This result can be attributed to the molecular rearrangements that occur during extraction under the influence of different parameters. Indeed, the process of extracting the essence of the raw material is delicate because some of the components are fragile and particularly sensitive to chemical transformations. These alterations occur after exposure to various conditions, such as ambient humidity, high temperature, radiation, oxygen, air,

and microbial agents **(Deschepper, 2017).** Terpenoids, in general, tends to be volatile and thermolabile which promote oxidation and hydrolysis reactions. The proximity of the structures of these aromatic compounds also facilitates conversion under particular distillation conditions. It is known that longer exposures to heat, humidity and more acidic pH conditions cause EO to undergo transformations **(Figueredo, 2007)**. The results clearly highlight that there is an increase in the amount of terpenic oxygen and a decrease in the amount of terpenic hydrocarbons with decreasing powder particle size. These results could be explained by the auto oxidation mechanism. In accordance with the findings of previous studies, grinding-induced decreases in particle size, increase the contact surface area of powders with atmospheric oxidants (hydroxyl radical OH, nitrate NO_3, and ozone O_3) and allow oxidation to be induced by light and oxygen. As a result, the EO auto oxidation process is more important for small powder particles than for large particles **(Noumi *et al*., 2021)**. Similarly, **Misra *et al*. (1996)** noted that a decrease in the size of powder particles increases air-oxygen, contact surface area, which favors oxygenation and hydroxylation reactions of carbon double bonds, favoring the transformation of terpenic hydrocarbons to terpenic oxygenated compounds. The degradability of aerobic terpenes varies according to the type of molecule and depends on the possible presence of a group. Alcohols are degraded at 99%, hydrocarbons at 75% and ketones at 12% **Wilson and Hrutfiord (1975).**

3.3. Antioxidant activity of the EO samples

DPPH free radicals absorb at 517 nm in their radical form. When these compounds were reduced by an antioxidant or a radical species, their absorption decreased. A lower absorbance of the reaction mixture indicates greater radical scavenging activity. The DPPH radical scavenging abilities of the different samples are shown in Table 4. The results showed that ascorbic acid had the lowest IC_{50} for DPPH radical reduction (13 ± 0.1). Moreover, among the fractions, the strongest activity was attributed to the EO in the ≤ 212 µm fraction, followed by that in the 212-425 µm fraction, which presented the lowest IC_{50} (146.12 ± 08; 78.2 ± 2.24) and therefore the best antiradical activities. Moreover, in the FRAP test, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The presence of antioxidants causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous (Fe^{2+}) form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm can be used to monitor the Fe^{2+} concentration. A higher absorbance indicates a higher reducing power **(Wei Cai Lee *et al*., 2012)**. After evaluating the reducing activity of different samples of EO through the FRAP test, by comparing the results obtained, we can conclude that the extracts of fine particles (212-425 µm and ≤ 212 µm) have the best reducing power (33.50 ± 0.40; 0.2 and 48.72 ± 0.028).

Table 4. Antioxidant activities of EOs from the different granulometric classes, mother powder, fresh and dry leaves of *L. chevalieri.*

Samples	DPPH IC_{50} (µg/mL)	FRAP (mgAAE/gEO)
FL	189.66 ± 1.6[ab]	21.20 ± 0.54[a]
DL	208.0 ± 0.9[a]	21.53 ± 0.33[a]
UP	182.13 ± 1.3[b]	27.95 ± 0.30[a]
≥ 425 µm	148.96 ± 1.8[c]	23.29 ± 0.40[a]
212-425 µm	146.12 ± 0.8[c]	33.50 ± 0.40[a]
≤ 212 µm	78.2 ± 2.24[d]	48.72 ± 0.028[b]
AA	13 ± 0.1[e]	-

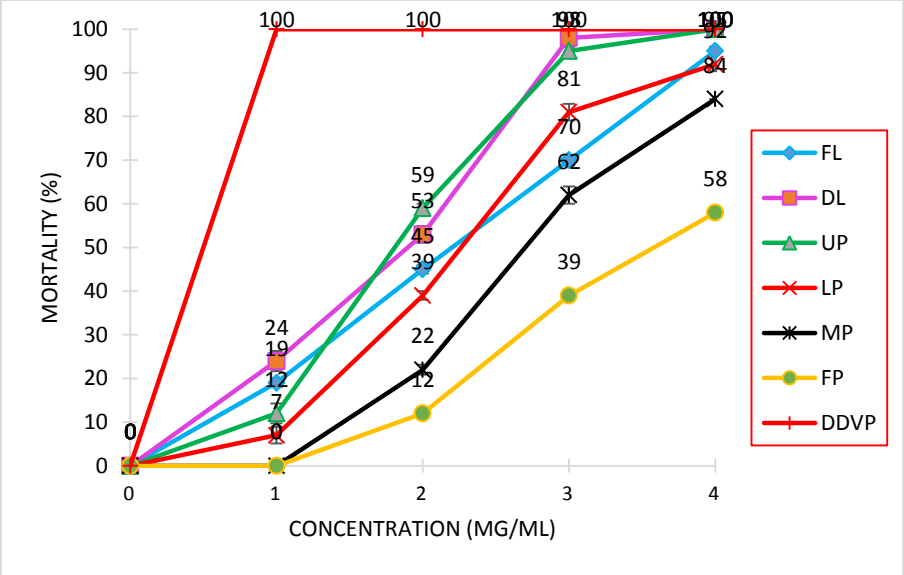
AAE: ascorbic acid equivalent. Means ± standard in the same column followed by different letters are significantly different (p < 0.05) according to Duncan's multiple range test (n = 3).

This result is in line with the work of **Noumi *et al.* (2021)**, which demonstrated that particle size fractionation concentrated most of the antioxidant molecules in the essential oils of fine particles, which would justify their strong anti-radicular and reducing capacities. In this study, we observed a particularly high content of oxygenated terpenes in the fine fraction, and the content decreases when the size of the fraction increases. Previous studies have revealed that carvacrol, an oxygenated terpene compound and the majority of EOs in the ≤ 212 µm fraction (over 65.77%), has a significant antioxidant effect **(Sokmen *et al*., 2004)**. According to **Dorman and Deans (2000)**, the molecular structure and the presence of oxygen in terpene molecules gives them greater antioxidant activity.

3.4. Effects of essential oils from different L. chevalieri fractions against An. gambiae larvae

Figure 2 presents the mortality percentage of *An. gambiae* larvae treated with different fractions of *L. chevalieri* EO. All the EOs exhibited insecticidal effects on *An. gambiae* larvae. The mortality rate was those dependent and varied depending on the fraction of the EO used. High activity with larval mortality percentages ranging from 12% at 1 mg/mL to 100% at 4 mg/mL was recorded with EOs from fresh leaves, dry leaves and unsieved powder of *Lippia chevalieri*. Large particles also had high mortality rates ranging from 7% at 1 mg/mL to 92% at 4 mg/mL. The lowest mortalities were obtained with median particles and fine particles, with mortality percentages ranging from 0% at 1

mg/mL to 84% at 4 mg/mL. A mortality rate of 100% was also registered for positive control (Dichlorvos) at the recommended single concentration of 1 mg/mL (1000 ppm), and a mortality rate less than 5% was recorded for the negative control.



FL: fresh leaves; DL: dry leaves; UP: unsieved powder**;** LP: large particles; MP: median particles; FP: fine particles; DDVP: dichlorvos

Figure 2: Mortality percentage of *Anopheles gambiae* larvae treated with different fractions of *L. chevalieri* EOs 24 hours post exposure.

Previous work assessing the larvicidal properties of EOs from *L. chevalieri* fractions has not been performed. However, some previous studies corroborate the larvicidal efficacy of *L. chevalieri* on several species of Culicidae. **Wangrawa *et al*. (2022a)** reported the significant larvicidal activity of fresh leaves of *L. chevalieri* against *Anopheles funestus* and *Culex quinque fasciatus*, with LC_{50} values of 54.11 and 105.74 ppm 24 hours post exposure, respectively; additionally, they reported a low mortality rate of less than 25% to 75 ppm against *Anopheles gambiae* larvae with the EO of *Lippia chevalieri* fresh leaves **(Wangrawa *et al.*, 2022b)**. The biological activity of plant EOs could be due mainly to the diversity and multitude of chemical compounds present in the plant. The major compounds found in different fractions of our plant, such as beta-cymene, thymol, carvacryl acetate, 1,3,8-p-menthane, terpinene, beta-myrcene, alpha-thujene, limonene, linalool, and geraniol, have been identified as having larvicidal properties **(Wangrawa *et al.*, 2022a; Youssefi *et al.*, 2019).** Secondary metabolites from plants causes physiological and cellular disturbances that include the inhibition of acetylcholinesterase, disruption of sodium and potassium ion exchange, and interference with mitochondrial respiration **(Younoussa, 2016)**. Moreover, they affect the midgut epithelium, gastric caecae and Malpighian tubules in mosquito larvae **(Younoussa, 2016).**

Table 5 below shows the LC_{50} and LC_{95} values (mg/mL) of *Lippia chevalieri* EO after 24 hours of exposure. Fresh leaves, dry leaves, unsieved powder and large particles induced significant ($P < 0.05$) concentration-dependent larvicidal activity, with LC_{50} values of 1.93, 1.55, 1.68 and 2.11 mg/mL, respectively. However, neither the median particles nor the fine particles induced a not significant ($P > 0.05$) larvicidal activity, with LC_{50} values of 2.70 and 3.53 mg/mL, respectively.

Table 5: LC_{50} and LC_{95} (mg/mL) values of *Lippia chevalieri* EOs fraction against *Anopheles gambiae* larvae 24 hours postexposure

Fraction	Slope±SE	R^2	LC_{50} (CI)	LC_{95} (CI)	χ^2
Fresh Leaves	3.67±0.17	0.99	1.93(1.75-2.10)	5.41(4.61-6.75)	44.93***
Dry Leaves	4.94±0.20	0.95	1.55(1.35-1.74)	3.34(2.83-4.29)	113.74***
Unsieved powder	5.90±0.23	0.94	1.68(1.58-1.78)	3.19(2.94-3.53)	33.71**
Large particles	5.00±0.20	0.94	2.11(1.99-2.22)	4.50(4.11-5.02)	27.63**
Median particles	6.14±0.29	0.91	2.70(2.62-2.78)	5.00(4.72-5.36)	5.44^{ns}
Fine paricles	4.82±0.29	0.90	3.53(3.39-3.69)	7.74(6.95-8.87)	4.74^{ns}

LC= lethal concentration; CI= confidence interval; R2= coefficient of determination; $\chi 2$= chi square; significance according to Tukey test (P=0.05); ^{ns}P>0,05; *P<0,05; **P<0,01; ***P<0,001;

EO extracted from the dry fraction and unsieved powder had greater larvicidal efficacy than EO extracted directly from fresh leaves, while EO extracted from the sieved fractions produced progressively less significant larvicidal activity depending on the particle size. This allows us to hypothesize that, drying and grinding increase plant efficacy, while fractioning fine particles progressively reduces efficacy as a function of particle size. This may be because the number and concentration of secondary metabolites responsible for larvicidal activity decreases with particle size.

4. CONCLUSION

Fractionation by sieving of *L. chevalieri* leaf powders enables the concentration of antioxidant terpene compounds in the EO of fine particles and, the improvement of the antioxidant capacity of the EO. Moreover, fractionation also reduces the chemical composition and hence, the larvicidal activity of the powder. This shows that the EO of *L. chevalieri* small-particle leaves is worthy of investigation in other potential disease-related assays. However, since the aim of quality extraction is to obtain an essential oil that is as faithful as possible to the original essence, it is important to have good control of different stages of extraction, which will limit the transformation of molecules.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Conceptualization, FOKO DADJI Gisele Aurelie, Djantou Elie Baudelaire, Yanou Njintang Nicolas, Tamesse Joseph Lebel and Dicko Amadou; Formal analysis, Rup-Jacques Sandrine; Investigation, FOKO DADJI Gisele Aurelie and Dainone Ignareki Damas; Methodology, FOKO DADJI Gisele Aurelie, Nsemi Muanda Francois and Rup-Jacques Sandrine; Project administration, Dicko Amadou; Resources, Djantou Elie Baudelaire, Nsemi Muanda Francois, Yanou Njintang Nicolas, Rup-Jacques Sandrine and Dicko Amadou; Software, FOKO DADJI Gisele Aurelie and Dainone Ignareki Damas; Supervision, Tamesse Joseph Lebel; Validation, Djantou Elie Baudelaire, Yanou Njintang Nicolas and Tamesse Joseph Lebel; Visualization, Yanou Njintang Nicolas; Writing – original draft, FOKO DADJI Gisele Aurelie; Writing – review & editing, FOKO DADJI Gisele Aurelie and Dicko Amadou..

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Availability of Data

The data used to support the findings of this study are available from the corresponding author upon request.

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