

Chemical composition, larvicidal and antibacterial activities of some leaves of the Cameroonians' flora

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<https://doi.org/10.51867/ajernet.6.3.35>

Submitted: 14th July 2025; Accepted: 31st July 2025; Published: 11th August 2025

ABSTRACT

Recent studies have demonstrated the resistance of target pests to certain chemical insecticides. Environmental pollution is one result of the use of these chemical insecticides. The objective of this study was to determine larvicidal and antibacterial properties of the essential oil extracted from plants (*Curcuma longa*, *Petroselinum crispum* and *Apium graveolens*) by hydrodistillation. These plants were selected because of their utilisation, for instance, as tea. Chemical composition was analyzed using gas chromatography (GC) and GC coupled with mass spectrometry (GC-MS). Larvicidal efficacy was assessed following guidelines established by the World Health Organization (WHO). *C. longa* yielded the highest extraction efficiency at 0.9%. All these samples are rich in monoterpenes (*C. longa*: 87.90%; *P. crispum*: 97.80%; *A. graveolens*: 88.08 %; *M. piperita*: 94.94%). In *C. longa* extract, Δ^3 -carène (37.40%) was the main compound. In the Apiaceae, 1,8-cinéole was the major component (*P. crispum*: 63.97% and *A. graveolens*: 63.33%). The larvicidal tests showed that *C. longa* and *P. crispum* exhibited the highest larvicidal activity on *Anopheles gambiae* s.l. larvae. The immature stages were most susceptible. In addition, *A. graveolens* and *P. crispum* exhibited excellent antibacterial properties. *Bacillus subtilis* was the most sensible (DI=12mm). *C. longa* and *P. crispum* essential oils can be used as bio-insecticide and *A. graveolens* as a natural antibiotic.

Keywords: *Anopheles gambiae*, *Bacillus subtilis*, Essential Oils, *Staphylococcus Warneri*

I. INTRODUCTION

The use of medicinal plants has seen a renewed interest in recent decades. This situation follows the high cost of synthetic molecules, their polluting effects on animals, humans, and the environment (Chandre *et al.*, 2000; Hargreaves *et al.*, 2000) and especially resistances observed in vectors and germs (Overgaard *et al.*, 2012). In Cameroon, for example, malaria, a parasitic disease whose vector is *Anopheles* mosquitoes, accounts for 40 to 50% of medical consultations, 23% of hospital admissions, and consumes approximately 40% of the annual household health budget. Economically, Cameroonian public authorities spend two to three billion CFA francs annually to combat this disease. Furthermore, resistance of the *Plasmodium* parasite and its vector to antimalarial drugs and synthetic insecticides (Carnavale & Mouchet, 2001) respectively has been demonstrated. Moreover, the application of insecticides pollutes the environment, posing a real risk of intoxication to non-target animal populations (Yang *et al.*, 2002; Kiran *et al.*, 2006; Senthikumar *et al.*, 2008). In this context, the use of plants and formulations derived therefrom is encouraged. Numerous studies highlighting the insecticidal activity of certain plant species from the Cameroonian pharmacopoeia have been conducted (Tchoumbougang *et al.*, 2008; Akono *et al.*, 2012). The use of plants possessing proven biological properties is further promoted. Plants from the Cameroonian pharmacopoeia represent an inexhaustible source of bioactive molecules that only need to be explored. Most naturopaths utilize them

in the treatment of gastroduodenal ulcers, intestinal inflammations, pancreatitis, rheumatoid arthritis, cough, umbilical hernia, as well as antibacterial agents (Dibong, 2015; Mpondo *et al.*, 2015).

Indeed, several studies have revealed that essential oils constitute the most effective plant formulation. It appears that the essential oils extracted from certain organs of plants belonging to these families possess excellent insecticidal (Chantraine *et al.*, 1998), antibacterial (Ruberto *et al.*, 2000), acaricidal (Lee, 2004), anti-inflammatory (Jazet *et al.*, 2009), antiparasitic (Nghang *et al.*, 2017), and antifungal (Lboumhamdi *et al.*, 2018) properties. Thus, a few plants from the Cameroonian flora have been identified to evaluate their larvicidal and antibacterial activities. This new formulation limits the risk of environmental pollution and enhances the improvement of population health.

II. METHODOLOGY

2.1 Plants's Leaves Harvest and Essentials Oils Extraction

Leaves of different plants species were collected from several orchards. According to the owners' opinions, these plots were free from any chemical insecticide treatment likely to influence the chemical composition of the volatile essences. After collection, the specimens were identified by teachers from the Department of Plants Organisms Biology at the University of Douala. This identification was then confirmed at the National Herbarium in Yaoundé. Leaves of the same plant species were placed in a bag and then transported to the Biochemistry Laboratory of the Faculty of Sciences at the University of Douala, where essential oils extraction took place. On site, the leaves were pre-cut, then washed with spring water. These leaves were weighed using a balance and then introduced into the flask of the hydro-distillation system. This latter process was the one used in this particular research. This method consists of directly immersing the plant material to be treated in a still filled with water, which is then brought to a boil. Hydro-distillation is performed using Clevenger-type apparatus during four hours. A quantity of water representing 2 to 3 times the mass of the plant material is introduced into the flask. The whole mixture is then brought to boil during four hours on the heating mantle. During heating, the bursting of leaf cells releases their active principles, which are carried by the water vapor. After condensation in the condenser, where cold water continuously circulates, a distillate consisting of two phases is obtained in the column: an aqueous phase that is constantly recycled and an odorous organic phase surmounting the first, consisting of essential oil. The distillation duration is 4 hours. Traces of water are removed by filtration through anhydrous sodium sulfate. The obtained essential oil is placed in dark glass bottles, then weighed and stored at a temperature of approximately 4°C. This method is considered to be reproducible and robust.

2.2 Chemical Composition Analysis by GC and GC/MS

A quantity of 10 µL of each essential oil sample was reserved for the determination of its chemical composition. This took place at the Faculty of Pharmacy of the University of Montpellier 2. The remaining fraction was intended for biological tests. Chemical analysis of the essential oils was performed using a Varian CP-3380 chromatograph equipped with a flame ionization detector and a capillary column (length 30 m, internal diameter 0.25 mm) with a non-polar stationary phase of the methylsilicone type (DB-1, film thickness 0.25 µm). Nitrogen was used as the carrier gas at a flow rate of 0.8 mL·min⁻¹. The injector temperature was 220°C; the detector was set at 250 °C. The oven was programmed from 50 °C to 200 °C with a temperature gradient of 5 °C·min⁻¹.

The retention indices of the different constituents were calculated relative to the retention times of a series of n-alkanes, and their relative percentages were calculated by electronic integration, considering that their response factors were all equal to 1. Gas chromatography-mass spectrometry coupling was performed using a Hewlett Packard HP 5970 A apparatus, equipped with a non-polar capillary column (30 m x 0.25 mm) of fused silica type HP-1 (film thickness 0.25 µm) and a quadrupole type detector (ionization energy 70 eV). The injector temperature was set at 220 °C and the interface zone temperature at 210 °C. Injection was performed in split mode (1:100) with 1 µL of a 10% essential oil solution in dichloromethane. The oven temperature was programmed from 70 °C to 200 °C at a rate of 10 °C·min⁻¹. Helium was used as the carrier gas with a flow rate of 0.6 mL·min⁻¹. Acquisition was performed in scan mode [35-300 amu] at 2.96 scan.sec⁻¹. The recorded chromatograms were then interpreted at the Biochemistry Laboratory of the University of Douala. The identification of the essential oils constituents was based on their retention indices and mass spectra by comparison with literature data and Adams (Adams, 2001). This method is also reproducible and robust.

2.3 Biological Tests

2.3.1 Larvicidal Tests

The larvicidal tests concerned larvae (immature and mature) of the *Anopheles gambiae* complex: laboratory *Anopheles gambiae* (Kisumu strain). The rearing of the *Anopheles gambiae* strain from OCEAC was conducted in the

bottle containing water maintained between 20°C and 30°C was introduced into a cooler at the insectarium of the University of Douala. In each 0.5 liter bottle, 25 larvae were introduced. Larvae were previously separated according to their developmental stages. Larvae of the same developmental stage were introduced into the same breeding tanks at a density of 100 larvae per 100 cL of water. The rearing water used was spring water drawn from around OCEAC. Larvae were then fed with a protein and mineral-rich powder, Tetrababy fish food (Desfontaines *et al.*, 1991) diluted in a small amount of water and poured into the tanks in variable proportions depending on their number and larval stages. For 100 larvae of stages I and II, 30 mg of powder were used per day, while 60 mg were necessary for stages III and IV. The obtained pupae were introduced into cups containing spring water and then placed inside cubic cages covered with mosquito netting. One side of each cage had an opening equipped with a sleeve through which various manipulations could be performed inside the cage. Adults emerging from the pupae were fed with a sugar solution soaked in cotton and introduced into the cage (Akono, 2010). These tests involved evaluating the mortality of *Anopheles* larvae in the presence of diluted essential oil solutions following a methodology inspired by the World Health Organization protocol (WHO, 1985).

Following this protocol, 20 immature stage larvae (L1, L2) were sampled using a Pasteur pipette and placed in 8 cm diameter bowls, each containing 99.9 mL of well water to which 0.1 mL of diluted essential oil was added. To do this, stock solutions of essential oils for each sample were prepared in 90% ethanol. Preliminary experiments allowed for the selection of a range of concentrations for the actual tests. From these, dilutions were made to obtain final experimental concentrations of 100, 150, and 200 ppm. Three repetitions were performed for each dilution. Two controls cups were also prepared under identical conditions to the test bowls. The negative control contained only ethanol (in the same proportions as for the trials, i.e., 1%) without any trace of essential oil. Larval mortality was counted every 5 minutes for 1 hour; then every hour for 10 hours, and finally after 24 hours of exposure to the volatile extracts solubilized in water. Larval mortality was detected when the larva showed no further movement, with or without a stimulus.

2.3.2 Antibacterial Tests

This test was diffusion discs method's or Kirby-Bauer methods. This method is standard and mostly reproducible. It was performed by depositing a sterile disc of 6 mm diameter impregnated with 25 µL of an essential oil solution (40 mg/mL concentration) on a Nutrient Broth agar-rich medium (Petri dish), previously inoculated with 0.2 mL of a bacterial solution with an optical density (OD=0.05-0.1). The essential oil was diluted in DMSO (Dimethyl sulfoxide), which is inert on antibacterial activity. 25 µL of gentamicin solution (500 µg/mL) was used as the reference antibiotic. After 24h of incubation at 37°C, results were read by measuring the diameters of the inhibition zones in millimeters (Chao *et al.*, 2000, Andrews *et al.*, 1980). The sensitivity of bacterial strains to the different essential oil was evaluated as follows: non-sensitive (-) for a diameter less than 6 mm; intermediate for a diameter between 6-8 mm; sensitive (+) for a diameter between 9-14 mm; very sensitive (++) for a diameter between 15-19 mm and extremely sensitive (+++) for a diameter of more than 20 mm (Ponce *et al.*, 2003)

III. FINDINGS & DISCUSSION

4.1 Extraction Yield

The extraction yield of *C. longa* (0.97%) was the highest, followed by that of *M. piperita* (0.17%) and *P. crispum* (0.15%), while the lowest yield was that of *A. graveolens* (0.14%). This difference in yield could be due to the fact that these species belong to different families (Table 1).

Table 1

Extraction Yield of Essential Oils from the Leaves of the Plants Studied

Plants				Essentials oils			
Families	Species	Organs	Weight (g)	Site	Color	Weight (g)	Yield (%)
Zingiberaceae	<i>C.longa</i>	leaves	1500	Ayos	Light yellow	14.58	0.97
Apiaceae	<i>P.crispum</i>	leaves	8200	Mbouda	Yellow	12.14	0.15
Apiaceae	<i>A.graveolens</i>	leaves	7200	Santa	Dark yellow	10.50	0.14
Lamiaceae	<i>M.piperita</i>	leaves	10500	Babadjou	Dark yellow	18.06	0.17

In comparison with data from the literature, it is noted that for the same plant species, there exists a difference in extraction yield depending on the collection site (Awasthi & Dixit, 2009; Essien *et al.*, 2017). This variation in yield could also be related to the plant organ from which the oil is extracted, the nature of the soil, the time of collection, the technique, and the duration required for the extraction (Vieira & Simon, 2000; Tchoumboungang *et al.*, 2008).

Furthermore, other factors may significantly influence this yield, such as the plant's developmental stage, pollination cycle, seasonal variations, and the plant's physiopathological condition (Tchoumboungang *et al.*, 2005; Probst, 2012).

4.2 Chemicals compositions of essentials oils

Table 2

Chemical Composition of the Essential Oils of the Leaves of C. longa, P. crispum, A.graveolens and M.piperita

Chemicals components	Percentages (%)				
	RI	<i>C. longa</i>	<i>P. crispum</i>	<i>A.graveolens</i>	<i>M.piperita</i>
Monoterpènes		87.90	97.8	88.08	94.94
MTH		76.55	33.72	24.74	16.21
α -thujene	924	-	2.31	0.35	-
α -pinene	934	3.55	-	0.17	1.53
Camphène	951	-	1.08	0.34	-
Sabinene	973	0.83	8.01	19.32	0.75
β -pinene	978	4.6	2.24	-	2.18
α-Myrcène	989	2.62	0.21	0.35	1.16
α -phellandrene	1000	-	0.16	4.07	-
Δ^3-carene	1010	35.40	6.16	-	-
α -terpinene	1017	0.96	-	-	0.45
p-cymene	1024	-	-	-	-
Limonène	1027	8.89	13.55	-	-
<Z>- β -ocimene	1033	18.52	-	-	5.95
<E>- β -ocimene	1047	-	-	-	0.27
γ -terpinène	1061	1.18	-	0.14	1.01
β -Guiaene	1431	-	-	-	2.79
α -Guiaene	1440	-	-	-	0.12
MTO		11.35	63.71	63.33	78.73
1,8-cinéole	1040.08	-	41.14	63.06	14.06
Terpinolène	1068	-	0.33	-	0.25
Linalol	1088	8.03	-	0.16	0.26
Oxyde d' α -pinène	1097	0.86	0.22	-	0.10
Oxyde de limonène	1120	0.02	0.08	-	-
Trans pinocarveol	1143	-	-	-	-
<trans-dihydro- α -> Terpineol	1144	0.07	-	-	-
Camphène hydrate	-	-	-	-	-
Borneol	1166	0.23	-	-	-
Menthol					0.31
Myrtenal	1173	-	0.29	-	-
<p> Cymen-8-ol	1179	0.49	-	-	-
Thuj-3-en-10-al	1181	-	0.13	-	-
<iso> Menthol	1182				1.85
α -terpinéol	1186	0.17	-	-	-
Dihydrocarveol	1192	0.58	-	-	-
<trans> Dihydrocarvone	1200	0.27	-	-	-
Neo-iso-menthol	1201	-	-	-	4.27
ρ -menthane	1232				1.01
Piperitone	1261	-	-	-	55.00
Géranol	1250	0.15	0.15	0.05	-
Géranial	1266	0.24	-	-	-
Limonène-10-ol	1288	-	-	-	0.11
Thymol	1289	0.08	-	0.06	0.13
Carvacrol	1299	-	0.14	-	0.13
Acetate de mytenyl	1317	0.16	0.17	-	-
Acetate de citronelyle	1326	-	-	-	0.74
Acetate de geranyle	1361	-	-	-	0.18
Acetate de geranyle	1363	-	21.06	-	-
Piperitone oxide	1364	-	-	-	0.33

Sesquiterpenes		10.27	2.56	11.52	3.38
STH		4.63	1.69	7.56	3.16
Bicycloelemene	1395	0.14	-	-	-
Z-caryophyllene	1396	-	-	-	1.14
γ-Elemene	1435	0.29	-	-	-
α-guaïène	1440	0.07	0.62	-	0.12
α -Humulene	1453	0.07	-	0.70	-
Caryophyllene-9-epi	1465	-	-	0.25	0.14
γ -Muuroène	1473	-	-	-	0.18
Zingiberene	1488	0.09	-	-	-
β -selinene	1489	0.58	-	-	1.27
Bicyclogermacrene	1496	1.86	1.07	3.59	-
α -farnesene	1506	-	-	3.02	0.31
α -Selinene	1509	0.28	-	-	-
Δ -cadinene	1527	1.25	-	-	-
α -Cadinene	1587	-	-	-	-
STO		5.64	0.87	4.06	0.22
Cubebol<10-epi>	1534	0.07	-	-	-
Spathulenol	1568	0.32	-	0.18	-
Cubenol	1593	0.26	0.52	-	-
Oxyde caryophyllene	1597	0.26	-	-	0.10
γ -Eudesmol	1617	0.33	-	-	-
Oxyde d' α -humelene	1624	-	0.35	2.05	0.12
α -Eudesmol	1630	0.42	-	1.03	-
γ -Muurololol	1634	0.12	-	-	-
α -Muurolol	1646	-	-	0.31	-
Calamenen-ol	1665	0.37	-	-	-
Bergamotol	1679	0.31	-	-	-
β -Cedrene epoxide	1622	-	-	0.49	-
β -Eudesmol	1649	-	-	-	-
β -Bisabolol	1673	3.08	-	-	-
α -Bisabolol	1687	-	-	-	-
<(Z)- α -trans>Bergamotol	1690	-	-	-	-

The results of the chemical analysis of these essential oils samples show that they are composed of monoterpenes and sesquiterpenes (Table 2).

The results indicate that the essential oil of *C. longa* has a predominance of hydrocarbon monoterpenes (76.35%) (Table 2); and its main constituent was Δ^3 -carene (35.40%). Furthermore, the analysis of the *Petroselinum crispum* and *Apium graveolens* essences samples shows that the main major compound was 1,8-cineole (41.14%) and (63.06%) (Table 2). Both are predominantly oxygenated monoterpenic. In contrast, the essential oil sample of *Mentha piperita* has piperitone (55.00%) as its predominant compound (Table 2). However, discrepancies with literature (e.g., Δ^3 -carene as characteristic of *C. longa*) should be acknowledged, emphasizing variability due to geographic or botanical factors. In literature data show that Δ^3 -carene is not always the characteristic compound of essential oils from *C. longa* plants species. As an illustration, α -Phellandrene was the major compound in the *C. longa* essential oil sample originating from Vietnam. It should therefore be suggested, in view of the above, that the essential oils of the plant's species from the Apiaceae family (*P. crispum* and *A. graveolens*) characterized in our study are of the 1,8-cineole chemical type. These results contrast with those found by (Petropoulos *et al.*, 2004) for *P. crispum* samples coming from Greece. In view of the above, it should be noted that the chemical composition of essential oils varieties depend on the species and botanical family.

4.2 Biological Activities

4.2.1 Larvicidal Activities

Biological tests show that the essential oils from the leaves of *P. crispum*, *C. longa* and *M.piperita* possess remarkable larvicidal properties on the immature and mature stages of *Anopheles gambiae* s.l (Table 3, and 5).

Table 3

Mortality percentage (%) of immatures larvae (I and II) of *Anopheles gambiae*(Kisumu) after 24h of exposition at différents ranges of concentrations of essentials oils (IC à 95%)

	Huiles essentielles	Concentrations (ppm)			
		200	150	100	Control
Mortality percentage of immatures larvae of <i>A. gambiae</i> s.l.	<i>M. piperita</i>	100,00 (100-100)	100,00 (100-100)	100,00 (100-100)	0 (0-0)
	<i>P. crispum</i>	100,00 (100-100)	100,00 (100-100)	100,00 (100-100)	0 (0-0)
	<i>A. graveolens</i>	100,00 (100-100)	96,67 (77,62-100)	71,67 (47,42-89,22)	0 (0-0)
	<i>C. longa</i>	100,00 (100-100)	100,00 (100-100)	100,00 (100-100)	0 (0-0)

Table 4

Mortality percentage (%) of matures larvae (III and IV) of *Anopheles gambiae*(Kisumu) after 24h of exposition at différents ranges of concentrations of essentials oils (IC à 95%)

	Essential oils	Concentrations (ppm)			
		200	150	100	Control
Mortality percentage of matures larvae of <i>A. gambiae</i> s.l.	<i>M. piperita</i>	100,00 (100-100)	100,00 (100-100)	93,33 (72,76-99,63)	0 (0-0)
	<i>P. crispum</i>	100,00 (100-100)	100,00 (100-100)	100,00 (100-100)	0 (0-0)
	<i>A. graveolens</i>	96,67 (77,62-100)	71,67 (47,42-89,22)	43,33 (21,72-66,98)	0 (0-0)
	<i>C. longa</i>	100,00 (100-100)	100,00 (100-100)	98,33 (80,26-100)	0 (0-0)

Table 5

Lethal Concentrations of Essential Oils (CL50 and CL95)

Species	Larvae stages			
	Immatures		Matures	
	CL50	CL95	CL50	CL95
<i>M.piperita</i>	< 89.00	<131.64	60.50	97.82
<i>P. crispum</i>	<89.00	<131.64	<44.93	<82.23
<i>A. graveolens</i>	92.43	133.61	111.40	203.95
<i>C. longa</i>	< 89.00	<131.64	44.93	82.23

The results demonstrate significant activity, especially in *C. longa* and *P. crispum*, aligning with known properties of major compounds. The discussion linking chemical constituents to toxicity is well-founded, with stage-specific sensitivity indicating practical implications for vector control. Larvicidal properties of *C. longa* essential oil could be due to its major compound Δ^3 -carene. Several studies have revealed that this molecule is an excellent insecticide. Indeed, the essential oil of Brazilian pepper, rich in Δ^3 -carene, was highly toxic to *Aedes aegypti*, *Culex quinquefasciatus*, and *A. gambiae* (Eggersdorfer, 2005). Regarding the Apiaceae family, the high content of 1,8-cineole (table 2) in the essential oils of the constituent plant species would explain their toxicity. This molecule has a neurotoxic action. It has been established that it is a natural pesticide with neurotoxic action that would act by inhibiting acetylcholinesterase activity, a substance that destroys acetylcholine after nerve impulse transmission (Huignard, 2013). Similarly, the work of (Chebli *et al.*, 2003; Vilela *et al.*, 2009) showed that pure 1,8-cineole caused inhibition of mycelial growth. The larvicidal activity in *M. piperita* (table 3) essence would be explained by the presence of piperitone (55%). This same compound isolated from *Mentha spicata* essence has demonstrated its larvicidal and ovicidal activity against *Anopheles stephensis* (Tripathi *et al.*, 2004). As for sensitivity depending on the developmental stages of mosquitoes, our work reveals that immature stages were significantly more sensitive than mature stages (table 3) (figure 1). This result would be inherent in the constitution of the larval integuments. Thus, the integuments of immature larvae being still in full growth (less rich in chitin) would allow easier diffusion of the essential oil compared to the integuments of mature larvae which would be significantly more constituted (Nkouandou *et al.*, 2020) his information is crucial for those responsible for the biological control program against malaria vectors. Indeed, it suggests interventions when larvae living in their breeding sites are still in immature stages. This would allow for interesting results with a negligible quantity of biocide (Figure 1).

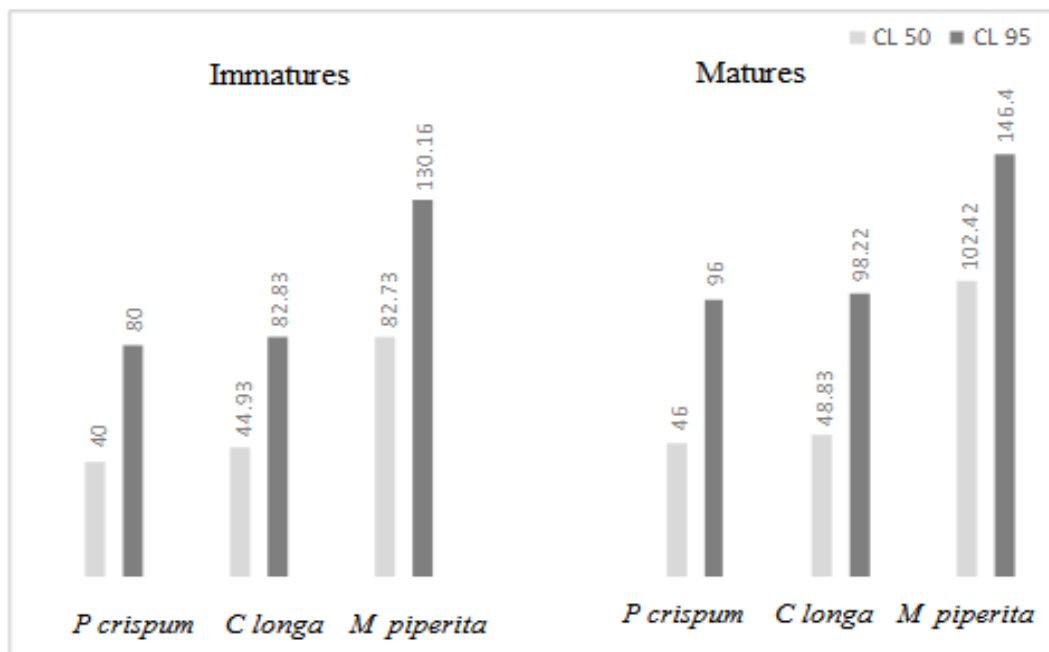


Figure 1
Larvicidal Activities

4.2.2 Antibacterials Activities

The essential oils induced diameters significantly smaller than those produced by gentamicin. Indeed, gentamicin induced the largest inhibition diameter (21 mm-23 mm), making the bacterial species extremely sensitive. These diameters varied depending on the oils used. The essential oils of *M. piperita* (7-10mm); *P. crispum* (7-10 mm) and *A. graveolens* (8-12 mm) exerted activity on the tested bacteria (table 4).

Tableau 4
Sensitivity of Different Bacteria Species Exposed to Essentials Oils

	Concentrations (µg/ml)	<i>Bs</i>	<i>Stw</i>	<i>Bs</i>	<i>Stw</i>
		Inhibition diameter [mm]		Sensitive	
Gentamycine	0.5	24	22	ES	ES
<i>M.piperita</i>	40	8	7	SI	SI
<i>A.graveolens</i>	40	12	8	S	SI
<i>P.crispum</i>	40	9	7	S	SI

NS= non sensitive SI= intermediary; S=sensitive ; HS=High sensitive; ES=Extremely sensible *Bs*: *Bacillus subtilis*; *Stw*: *Staphylococcus warneri*

The oils show moderate activity, with inhibition zones smaller than gentamicin, indicating potential but limited antibacterial efficacy. Antibacterial activity showed that the essential oils from fresh leaves of *M. piperita*, *A. graveolens*, and *P. crispum* were the most active (table 4, figure 2 and figure 3). They significantly inhibited all tested bacterial strains. This efficacy would be due to the presence of certain bioactive molecules contained in these oils. Clear presentation of data supports the conclusion that certain oils are active, possibly due to compounds like 1,8-cineole and piperitone (Table 4, figure 2 and figure 3).

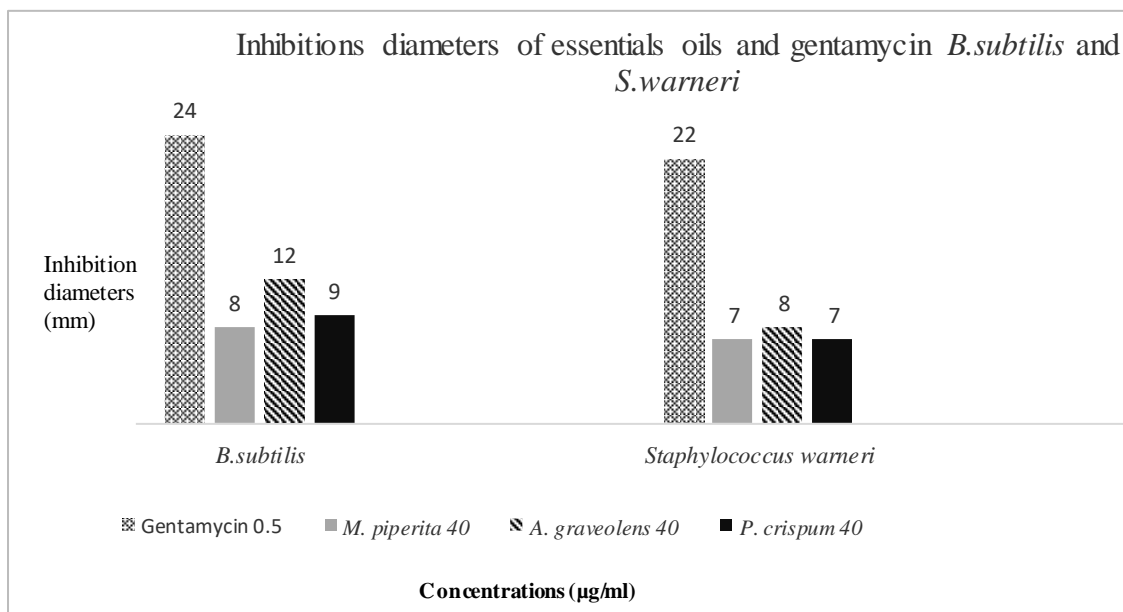


Figure 2
Inhibitions Diameters

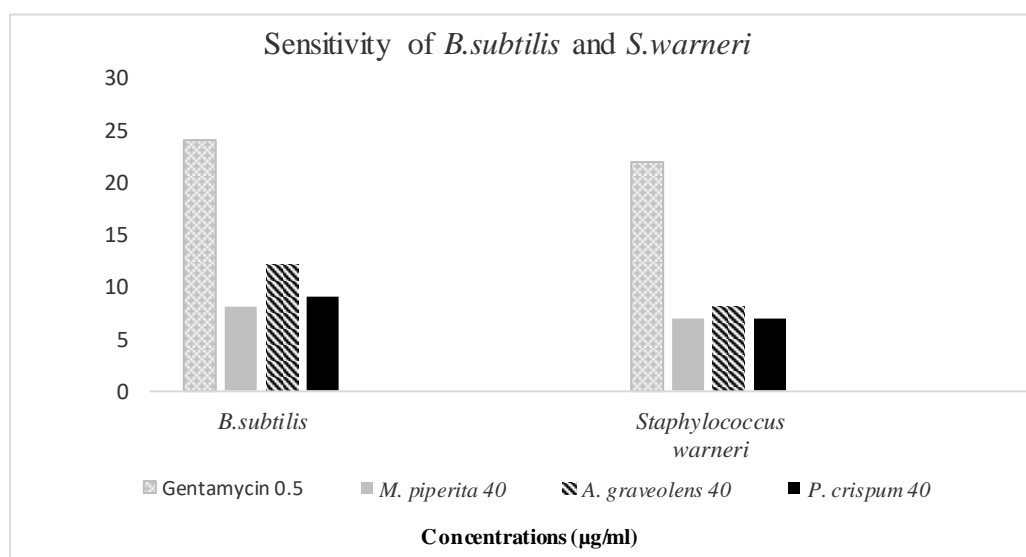


Figure 3
Sensitivity of Bacteria (Bacillus subtilis and Staphylococcus warneri) Exposed to Essentials Oils

V. CONCLUSION & RECOMMENDATIONS

5.1 Conclusion

This study had the objective to demonstrate the larvicidal and antibacterial activities to *C. longa*, *P. crispum*, *A.graveolens* and *M. piperita*. The essentials oils of *C. longa* and *P. crispum* were most active. These can be preselected for the formulation of bio-insecticides. Similarly, that of *A. graveolens* can be used for a natural antibiotic formulation.

5.2 Recommendations

This study recommends promoting research focused on natural molecules and plant-based formulations, which constitute indispensable alternatives to synthetic molecules responsible for numerous health and environmental problems.

Conflict of interest

This work does not have any conflict of interest

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