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RESEARCH

Molluscicide Effects of *Curcuma longa* L. Essential Oil Nanoemulsions on *Biomphalaria glabrata*

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Abstract

Schistosomiasis is a neglected tropical disease associated with poor sanitation and caused by parasitic trematodes of the genus *Schistosoma*. Its transmission cycle involves freshwater snails of the *Biomphalaria* genus as intermediate hosts. Niclosamide is recommended by the World Health Organization (WHO) for controlling *Biomphalaria* spp. populations, and concerns about its environmental toxicity and resistance highlight the need for the development of new alternatives. *Curcuma longa* L. essential oil is reported to have antimicrobial and insecticidal properties, suggesting its potential for application in mollusk control strategies. However, its lipophilicity limits its application in aqueous environments. This study aimed to develop *C. longa* nanoemulsions and evaluate their molluscicidal activity toward *Biomphalaria glabrata*. The essential oil, obtained by hydrodistillation, was characterized by GC–MS and GC–FID, revealing ar-turmerone (38.72%), turmerone (27.94%), and curlone (25.38%) as major components. Nanoemulsions prepared via the low-energy method with polysorbate 80 as a surfactant presented a droplet size of 11.8 ± 0.25 nm and a polydispersity index (PdI) of 0.183 ± 0.028 and remained stable between 25 and 45 °C. The polysorbate 20 nanoemulsion had a droplet size of 115.9 ± 0.72 and a PdI of 0.209 ± 0.010 and remained stable up to 50 °C. Molluscicidal assays with the nanoformulations followed WHO protocols. The LC_{90} values ranged from 44.84 to 89.72 µg/mL and from 33.86 to 126.42 µg/mL for the nanoformulations containing polysorbate 80 and polysorbate 20, respectively. The nanoemulsion formulated with polysorbate 80 showed promising molluscicidal activity against *Biomphalaria glabrata*, since its values remained within the efficacy criteria established by the World Health Organization (OMS), which recommends an LC_{90} of less than or equal to 100 µg/mL. These results highlight the potential of nanoemulsions as environmentally friendly alternatives to assist in schistosomiasis control programs, especially through the control of intermediate hosts involved in disease transmission.

Keywords Schistosomiasis · Intermediate host · *Biomphalaria glabrata* · Natural products · *Nanoemulsion* · *Molluscicidal activity*

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1 Introduction

Schistosomiasis is a neglected parasitic disease that can present in both acute and chronic forms and is caused by trematodes of the genus *Schistosoma* [1]. Transmission begins with the release of parasite eggs in human feces, which, upon reaching freshwater environments, hatch and release miracidia. These larvae penetrate snails of *Biomphalaria* sp., which act as intermediate hosts. The second infective larval stage (cercariae) takes place within these mollusks [2]. After being released into the water, cercariae actively penetrate human skin upon contact with the host, completing their life cycle by differentiating into adult forms inside the organism [3].

Owing to the complexity of its transmission cycle and its strong association with inadequate sanitation and exposure to contaminated freshwater, schistosomiasis remains a major public health issue worldwide [4]. The disease is present in 78 countries, and in Brazil, approximately 25 million people are estimated to live in areas at risk of infection, particularly in regions with poor sanitation [5]. Globally, more than 250 million people are currently infected, with approximately 280,000 deaths per year [6]. In endemic areas, continuous exposure to contaminated environments facilitates reinfection, potentially leading to a progressive increase in parasite load in the absence of treatment, making schistosomiasis a serious public and economic health concern [7].

As a complementary strategy, schistosomiasis involves the control of intermediate hosts, especially in areas with high prevalence rates or during outbreaks of acute cases [8]. This control is carried out via the use of niclosamide, a synthetic molluscicidal agent with well-documented environmental toxicity [9]. Its molluscicidal activity is associated with disruption of aerobic respiration and mitochondrial oxidative phosphorylation, resulting in impaired energy production and ultimately leading to mollusk death [4, 10]. The low selectivity of this compound, coupled with its potential to induce resistance in *Biomphalaria glabrata*, highlights the need for alternative molluskicidal agents that are eco-friendly and nontoxic to nontarget organisms [11].

In this context, plant-derived products have emerged as promising sources of biologically active compounds produced through specialized metabolism [12]. Among the natural products investigated as molluscicidal agents against *Biomphalaria glabrata*, notable examples include the aqueous stem extract of Serjania spp. ("cipó-timbó"), the latex of *Euphorbia milii var. hislopii*, and the essential oil of *Cymbopogon winterianus*, all of which have demonstrated potential in the control of this mollusk species [13].

In this study, we investigated the species *Curcuma longa L.*, a member of the Zingiberaceae family known for its broad pharmacological potential and diverse applications. Its biological activities are associated mainly with compounds that exhibit anti-inflammatory, antimicrobial, antifungal, anthelmintic, and notably antioxidant properties [14]. Native to the tropical forests of India, *C. longa* has rhizomes rich in curcuminoids that are responsible for its characteristic yellow coloration [15]. The essential oils extracted from these rhizomes are composed primarily of monoterpenes (such as terpinolene, 1,8-cineole, p-cymene, and phellandrene) and oxygenated sesquiterpenes (such as aR-curcumene, α-zingiberene, and β-sesquiphellandrene), which exhibit a variety of biological activities, including antimicrobial and insecticidal effects [16].

Despite their potential, the application of essential oils is limited by their high lipophilicity, which hinders their dispersion in aqueous media [17]. Nanotechnology presents an effective strategy to overcome this limitation by enabling the incorporation of essential oils into water-based systems through the reduction of droplet size to the nanometer scale (20–200 nm) [18]. Nanoemulsions are kinetically stable yet thermodynamically unstable systems in which one liquid is dispersed within another immiscible liquid [19]. Unlike conventional emulsions, the high surface area-to-volume ratio characteristic of nanoemulsions enhances the bioavailability and biological efficacy of encapsulated compounds by facilitating their interaction with target organisms in aquatic environments since this geometric modification enhances mass transfer kinetics and the dissolution rate of encapsulated compounds [20]. Furthermore, the high curvature of the droplets induces a rise in saturation solubility, which facilitates the partition of active compounds into the target organism's membranes [21]. Moreover, in aquatic environments, a small droplet size ensures superior kinetic stability; the predominant Brownian motion counteracts gravitational forces, effectively preventing phase separation processes such as sedimentation or creating and maintaining formulation homogeneity in water systems [21].

These systems generally require surfactants to maintain stability and are typically composed of an aqueous phase and an oil phase [22]. In this context, the choice of an appropriate surfactant, on the basis of its chemical properties, affinity with the oil, and ability to reduce interfacial tension, is a determining factor in nanoemulsion development to ensure long-term stability [23]. The selection of the surfactant system is critical to ensure the thermodynamic stability and functionality of the nanoemulsions. In this context, polysorbates are widely used because of their nonionic characteristics, which confer high resistance to variations in pH and ionic strength, in addition to minimizing undesirable electrostatic interactions in complex matrices [24]. The high hydrophilic–lipophilic balance (HLB) values of these compounds favor the formation of oil-in-water (O/W) systems with reduced droplet diameters. From a regulatory

and toxicological perspective, polysorbates are preferred for their high biocompatibility, biodegradability, and generally recognized as safe (GRAS) status by international agencies such as the FDA, which guarantees their applicability in biological systems [25].

2 Methodology

2.1 Plant Material

The *Curcuma longa* rhizomes were collected at Lumiar, São Pedro da Serra, Rio de Janeiro state, Brazil (coordinates − 22.35471794247414, -42.34565350855477). Research on the plant material was authorized by SISGen (A491A56).

2.2 Essential Oil Extraction

Curcuma longa essential oil from fresh rhizomes (1290.3 g) was obtained by hydrodistillation in a modified Clevenger apparatus for 3 h. To do this, the rhizomes were cleaned with water to remove dirt, after which the rhizomes were crushed in distilled water in a blender (model SPL-052; Spolu-Benesse, Itajobi, SP, Brazil), added to a 5 L round-bottom flask, and subjected to extraction. After that, the essential oil was dried with sodium sulfate (Na_2SO_4, ≥ 99%, Sigma–Aldrich, St. Louis, MO, USA) and stored in a 5 mL amber glass flask with polypropylene caps at -20 °C.

2.3 Essential Oil Characterization

For essential oil chemical characterization, a GC–MS QP2010 (Shimadzu, Kyoto, Japan) gas chromatograph equipped with a mass spectrometer was used to identify the components, and a GC-2014 (Shimadzu, Kyoto, Japan) gas chromatograph equipped with a flame ionization detector (FID) was used to determine the relative abundances. One microliter of the oil, dissolved in dichloromethane at 1000 ppm, was injected with a 260 °C injector. The carrier gas was helium at a flow rate of 1 mL/min with split injection (1:40). The column was an RTX5 for MS (RESTEK Corporation, Glastron, FR, 0.25 mm ID, 30 m in length, 0.25 µm). The oven temperature was initially 60 °C and then increased to 290 °C at a rate of 3 °C/min. The mass spectrometry conditions included 70 eV electron ionization and 1 scan/s. The FID chromatographic conditions were the same except that the FID temperature was 290 °C. The arithmetic index (AI) was calculated by interpolating the retention times of a mixture (C7–C40) of aliphatic hydrocarbons (Sigma–Aldrich, St. Louis, MO, USA) analyzed under the same chromatographic conditions as the sample. The identification of the substances was performed by comparing their AI and mass spectra with those reported in the literature [26, 27] and NIST mass spectrum libraries. The FID peak area normalization method was used to determine the relative abundance of chemical constituents.

2.4 Nanoemulsion Preparation

The nanoformulations were prepared via phase inversion via composition via the low-energy method. The oil phase was composed of 1% *Curcuma longa* essential oil from fresh rhizomes and 9% surfactant polysorbate 20 (HLB 16.7) or polysorbate 80 (HLB 15). The aqueous phase consisted of 90% deionized water. First, the oil phase (essential oil and surfactant) was homogenized in a vortex (model 56AP, Phoenix), and then the aqueous phase (deionized water) was slowly dripped into the oil phase under constant homogenization.

2.5 Nanoemulsion Characterization

The formulations were macroscopically evaluated for appearance, with translucent behavior and the absence of phase separation considered ideal. Nanometrically, the average droplet size (nm) and polydispersity index were characterized via dynamic light scattering (DLS) via a Zetasizer Pro Blue (Malvern, UK) at various time points throughout the day of preparation, 24 h later, and after 7 and 15 days. All analyses were performed in triplicate.

2.6 Nanoemulsion Stability

The nanoemulsions were subjected to thermal stress to evaluate their stability under increasing temperatures (25 °C to 60 °C) in a NanoSizer S90 (Malvern, UK). The mean droplet size (nm) and polydispersity index were characterized as described in Sect. 2.5, with measurements taken at 10 °C intervals.

2.7 Molluscicidal Activity Assay

The experiments were carried out according to the methodology of SANTOS et al. [28], where juveniles (3–5 mm and 6–8 mm) and adults (10–12 mm shell diameters) of the species *B. glabrata*, not infected by *S. mansoni*, were individually allocated to a 24-well plate. The mollusks were collected in the breeding tank at the Lauro Travassos Pavilion at the Oswaldo Cruz Institute - IOC/Fiocruz. The snails were exposed to negative controls (distilled water, P20 blank and P80 blank), positive controls (niclosamide 2 mg/L), and different concentrations of *C. longa* essential oil nanoemulsion to a final volume of 2 mL per well. In this

Table 1 Chemical characterization of the essential oil from *Curcuma longa* L. rhizomes via GC–MS and GC–FID

	RT	AI_{rep}	AI_{exp}	Substances	Relative abundance (%)
1	8.681	1002	1007	α-phellandrene	0.89
2	9.324	1022	1024	o-cymene	1.09
3	9.594	1046	1031	1,8-cineole	0.63
4	28.123	1479	1477	α-curcumene	2.43
5	28.693	1493	1491	α-zingiberene	1.04
6	29.838	1521	1520	β-sesquiphellandrene	1.87
7	35.171	1670	1661	ar-turmerone	**38.72**
8	35.365	1674	1666	Turmerone	**27.94**
9	36.560	1697	1699	Curlone	**25.38**
TOTAL					99.9
Monoterpene hydrocarbon					0.89
Oxygenated monoterpene					1.72
Sesquiterpene hydrocarbons					5.34
Oxygenated sesquiterpene					72.95

*RT retention time; AI_{Lit} arithmetic index from the literature; AI_{Exp} arithmetic index calculated

bioassay, the snails were exposed to the media for 24 h. The experiments were carried out in triplicate and on at least 3 different days. The nanoemulsions were tested at concentrations ranging from 10 to 120 µg/mL (expressed in essential oil). Mortality was observed within 24 h, and mortality was affected by immobility, shell depigmentation, hemolymph release, absence of a heartbeat, and/or exposure to the visceral mass [29, 30].

3 Results

3.1 Essential Oil Extraction and Characterization

The essential oil content of *C. longa* rhizomes was 0.62%. Table 1 shows the chemical characterization of the essential oil, with the major fraction composed of oxygenated sesquiterpenes comprising 72.95% of the oil. In addition, the major constituents found were ar-turmerone (38.72%), turmerone (27.94%), and curlone (β-turmerone, 25.38%) (Fig. 1).

3.2 Nanoemulsion Formulation and Characterization

The evaluations were conducted at five time intervals after preparation (0, 30, 60, 90, and 120 min) and on four distinct days after preparation (0, 1, 7, and 15). On the day of preparation, the nanoemulsion containing polysorbate 20 (P20) exhibited a transparent and homogeneous appearance (Fig. 2a). Shortly after, a gradual increase in turbidity was observed, leading to the formation of a translucent system with a bluish reflection, characteristic of the Tyndall effect [31]. Over time, progressive droplet growth and increased polydispersity were noted. After 24 h (Fig. 3a), the system remained macroscopically stable, with no visible signs of destabilization, such as phase separation, creaming, or sedimentation. At the nanometric level, the mean droplet size increased from 11.11 nm to 72.95 nm on the first day. On subsequent days, this parameter fluctuated within a range of approximately ± 10 nm, reaching 60.98 nm after 15 days (Fig. 4a and Fig. 5a).

In contrast, the formulation containing polysorbate 80 (P80) exhibited a translucent appearance with a bluish reflection immediately after preparation (Fig. 2b). Over

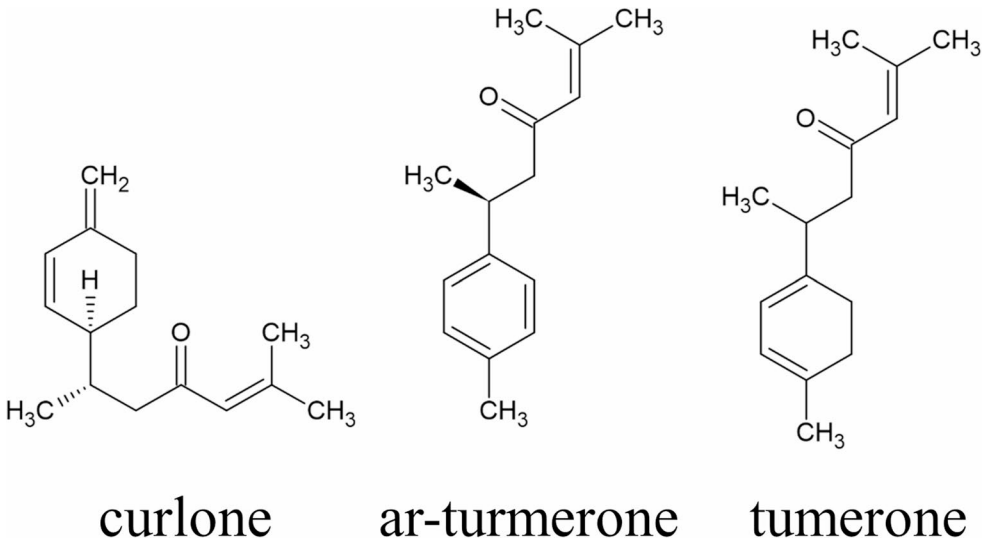


Fig. 1 Major constituents of *Curcuma longa* L. essential oil from fresh rhizomes

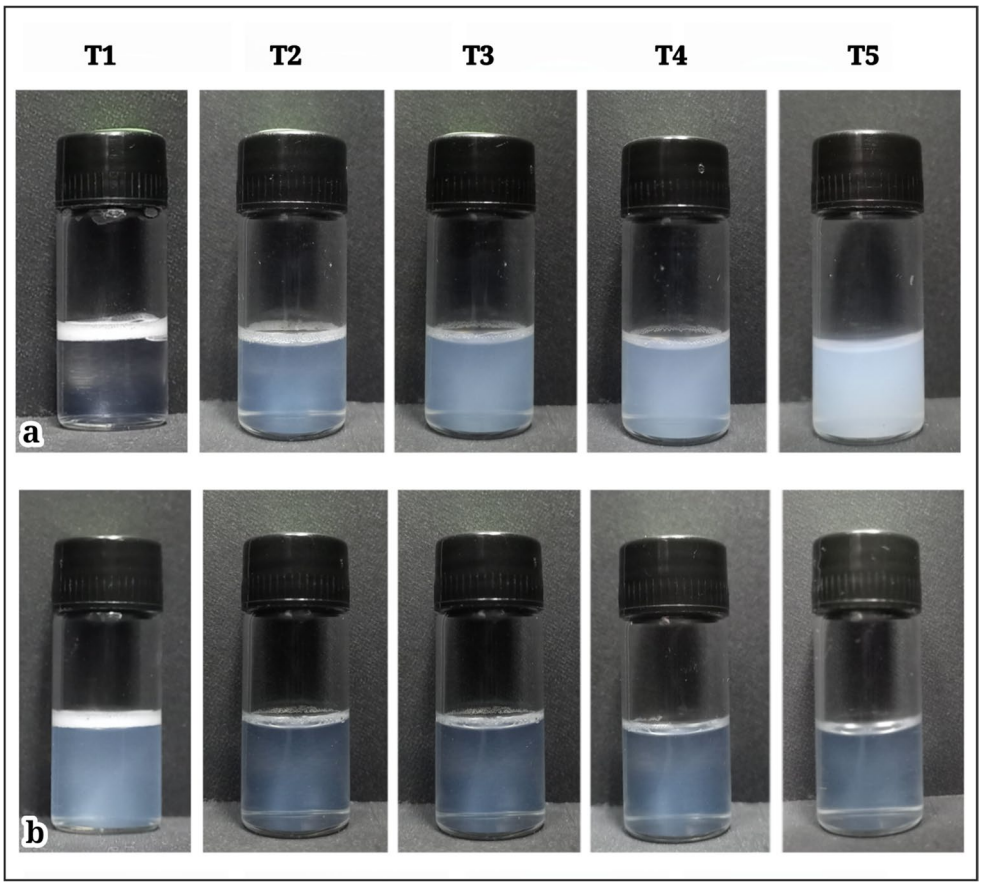


Fig. 2 Nanoformulations on day 0 of preparation at different times (T1–T5: 0, 30, 60, 90, and 120 min): (**a**) nanoformulation with *C. longa* essential oil and polysorbate 20; (**b**) nanoformulation with *C. longa* essential oil and polysorbate 80

time, these visual characteristics gradually decreased, resulting in a more transparent system after 24 h of storage (Fig. 3b). No macroscopic signs of instability, such as phase separation or flocculation, were observed throughout the evaluation period. Despite exhibiting a bimodal nanometric profile, this behavior remained stable on the first day of analysis, with only slight variations in the mean droplet size (38.28 to 31.72 nm). Over 15 days, a gradual migration of droplet populations toward a micellar distribution was observed, resulting in a monomodal profile and a mean size of 12.04 nm (Fig. 4b and Fig. 5b).

Detailed measurements of the droplet size distribution, polydispersity index, and nanoscale colloidal behavior are provided in Tables 2 and 3.

3.3 Nanoemulsion Thermal Stability

The particle diameter (nm) and polydispersity index (Table 4) and colloidal behavior of the polysorbate 20 (P20) nanoemulsion were maintained at 50 °C (Figs. 6 and 25–50 °C), indicating a monomodal and monodisperse distribution. However, at temperatures exceeding 50 °C, the P20 nanoemulsion exhibited a significant ($p<0.001$) increase in droplet size. At 55 °C, 56.7% of the droplets grew, and at 60 °C, 180% of the droplets grew (Eq. 1). This resulted in the emergence of multiple droplet populations, as illustrated in Fig. 2 (25–60 °C). These observations suggest that the colloidal system is becoming increasingly unstable. Furthermore, the macroscopic characteristics of the nanoemulsion changed at 55 °C, indicating a transition from an initial bluish translucent appearance to a milky-white appearance.

$$Growth\ percentage\ (\%) = \left(\frac{Md1 - Md0}{Md0}\right) \times 100 \qquad (1)$$

Md1 Final mean droplet size (nm).
Md0 Initial mean droplet size (nm).

In contrast, the *C. longa* nanoemulsion formulated with polysorbate 80 (P80) displayed monomodal behavior, with an average particle size of 12.18 ± 0.04933 nm and an intensity of 97.8%, which is indicative of micellar dispersion, when it was subjected to temperatures up to 45 °C (p value of 0.9976). Upon a temperature increase of 5 °C, a second droplet population begins to emerge, leading to a transition in colloidal behavior (p value of 0.0031). At 55 °C, the intensity of the initial micellar population decreased to 9.3%, whereas the second population, characterized by a diameter of 101.8 nm, exhibited an intensity of 90.7%. At 60 °C, a single population with an average diameter of

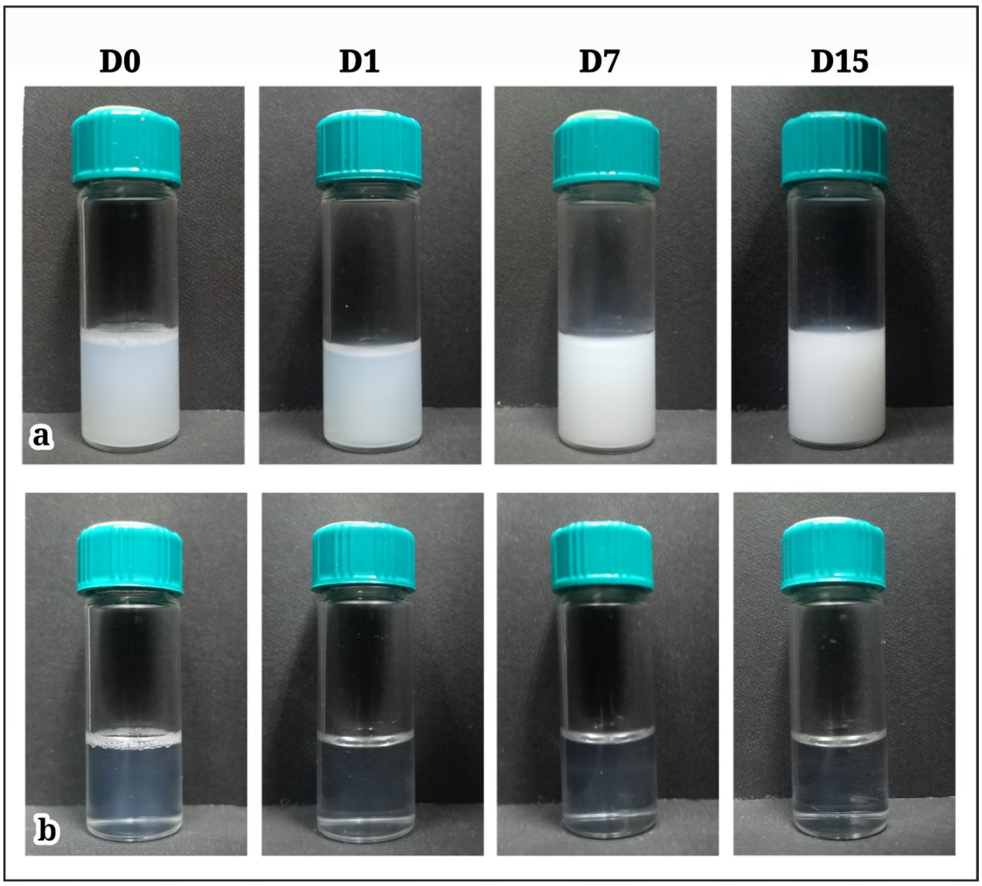


Fig. 3 Nanoformulations on day 0 of preparation and after 1, 7, and 15 days: (**a**) nanoformulation with *C. longa* essential oil and polysorbate 20; (**b**) nanoformulation with *C. longa* essential oil and polysorbate 80

92.16 ± 2.203 nm and a PdI of 0.224 ± 0.018 presented a relative intensity of 100% (Fig. 7). In terms of the macroscopic characteristics of the P80 formulations, a transition from a transparent to a bluish translucent liquid was observed after thermal exposure.

3.4 Molluscicidal Activity Assay

The nanoemulsions resulted in mortality rates of 100 ± 0% at 120 µg/mL and 0 ± 0% at 10 µg/mL in juvenile *Biomphalaria glabrata* samples, with 3–5 mm shell diameters for both nanoemulsions (P20 and P80). For mollusks measuring 6 to 8 mm in length, the P20 and P80 nanoemulsions had mortality rates of 88.8 ± 19.2% at concentrations of 120 and 100 µg/mL, respectively. In the case of adult mollusks (10 to 12 mm), the P20 and P80 nanoemulsions resulted in 100 ± 0% and 88.8 ± 19.24% mortality, respectively, at 120 µg/mL. The positive control (niclosamide 2 µg/mL) resulted in 100 ± 0% mortality during all growth stages (3–5 mm, 6–8 mm, and 10–12 mm) of *Biomphalaria glabrata*. With respect to the negative control (distilled water) and the nanoemulsion blanks P20 and P80 (without essential oil), no mortality was observed after 24 h. The mortality data can be found in Table 6.

The median lethal concentrations ($_{LC50s}$) of the polysorbate 20 and polysorbate 80 nanoemulsions were 19.65, 53.65, and 68.44 µg/mL and 22.39, 28.90, and 52.14 µg/mL, respectively, for juvenile (3–5 and 6–8 mm) and adult (10–12 mm) *Biomphalaria glabrata* mollusks (Table 5). The LC_{50} and LC_{90} of the polysorbate 20 and 80 nanoemulsions are shown in Table 3 and 6.

4 Discussion

The essential oils of *Curcuma longa* are widely documented in the literature, with oxygenated sesquiterpenes as the predominant fraction. The most common constituents found in essential oils derived from rhizomes include α-turmerone, β-turmerones, and aR-turmerones [13, 25]. In this study, the results of the essential oil analysis corroborated those of previous reports, identifying ar-turmerone (38.72%), turmerone (27.94%), and curlone (25.38%) as the major compounds. Additionally, the oil contained characteristic minor compounds such as 1,8-cineole, α-curcumene, α-zingiberene, o-cymene, β-sesquiphellandrene, and α-phellandrene [32].

The colloidal behavior observed in the nanoformulations containing *C. longa* essential oil may be influenced by its

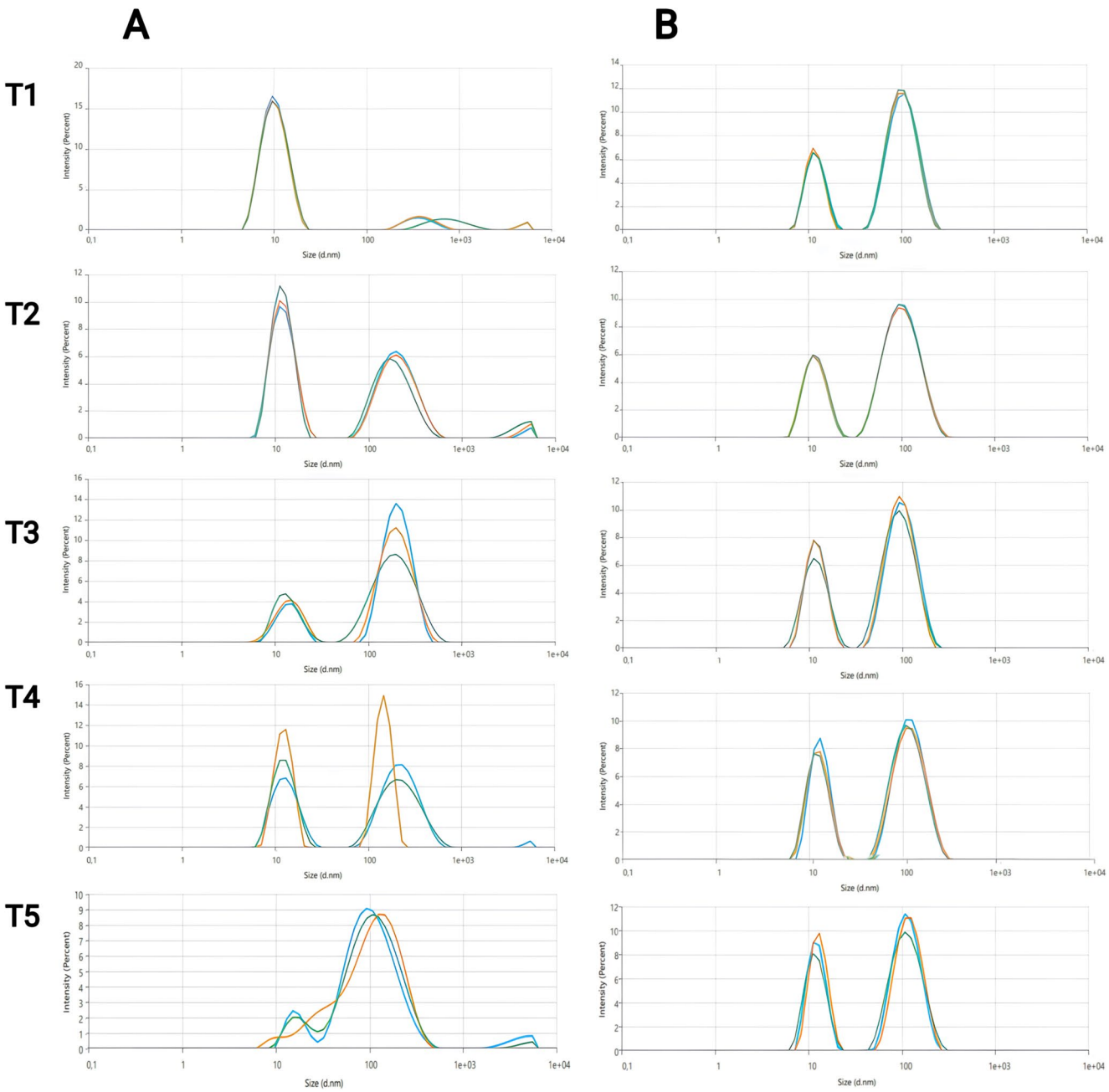


Fig. 4 Size distribution by intensity of droplets of *C. longa* essential oil nanoemulsions on day 0 of preparation at different times (T1–T5: 0, 30, 60, 90, and 120 min): (**A**) nanoemulsion with polysorbate 20; (**B**) nanoemulsion with polysorbate 80

chemical complexity and physicochemical properties, since the oil contains a variety of sesquiterpenes and oxygenated compounds (e.g., ar-turmerone, turmerone, and curlone), which confer moderate to low polarity and relatively high lipophilicity. Polysorbate 20 (P20), HLB 16.7; LogP 2.32, is structurally composed of a short (C12) lauric acid chain, which gives it a more hydrophilic character than polysorbate 80 (P80), HLB 15.0; LogP 4.99, which contains a long (C18), unsaturated oleic acid chain, conferring greater affinity for heavier and less polar oils [33].

In this context, P20 may have promoted greater initial dispersion and apparent solubilization of the essential oil in the aqueous phase, resulting in a transparent system immediately after preparation. However, this higher affinity for the aqueous phase may have led to the formation of a less flexible interfacial film around the oil droplets, compromising long-term stability and causing a rapid increase in turbidity, mean droplet size, and polydispersity [34]. In contrast, although P80 initially produced a translucent formulation with a bluish reflection, it became progressively more

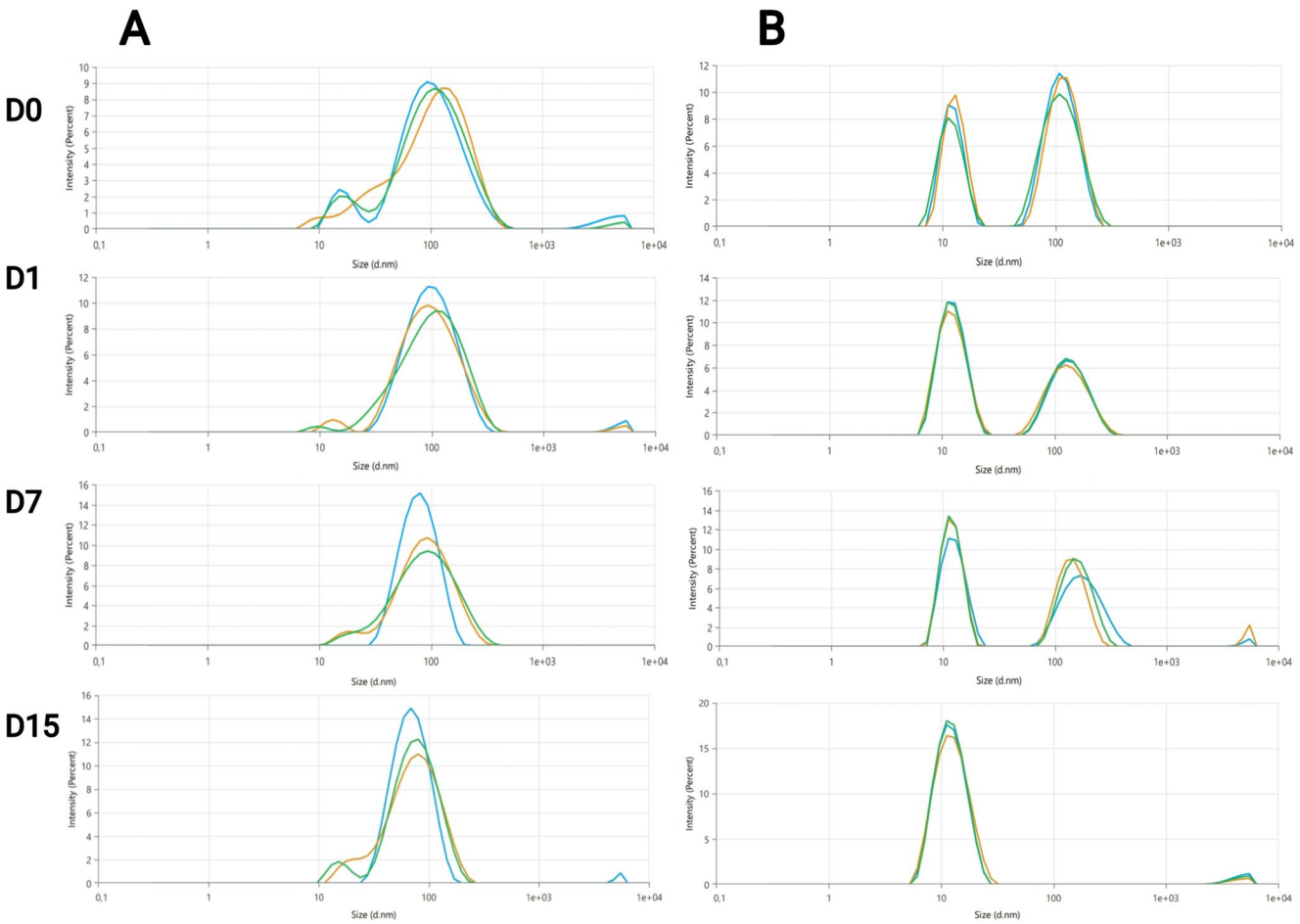


Fig. 5 Size distribution by intensity of droplets of *C. longa* essential oil nanoemulsions after 0, 1, 7, and 15 days of preparation: (**A**) nanoemulsion with polysorbate 20; (**B**) nanoemulsion with polysorbate 80

Table 2 Mean droplet size (nm) and polydispersity index (PDI) of the P20 and P80 nanoemulsions prepared with *C. longa* essential oil after preparation (day 0) at different times: T1–T5 (0, 30, 60, 90, and 120 min)

Time (min)		Polysorbate 20 (A)			Polysorbate 80 (B)		
		Z-Average (d.nm)	Polydispersity index	Zeta potential (mV)	Z-Average (d.nm)	Polydispersity index	Zeta potential (mV)
T1	0	11.11 ± 0.20	0.28 ± 0.02	-19.74 ± 0.57	38.28 ± 0.16	0.60 ± 0.00	-10.25 ± 0.97
T2	30	23.50 ± 0.53	0.72 ± 0.02	-16.69 ± 0.96^{c}	36.63 ± 0.31^{e}	0.58 ± 0.01	-10.17 ± 4.24
T3	60	60.67 ± 16.4	0.91 + 0.15^{b}	-9.31 ± 1.83^{d}	30.65 ± 0.40^{d}	0.57 ± 0.01	-9.49 ± 2.23^{d}
T4	90	62.43 ± 54.44	0.67 ± 0.02	-8.43 ± 0.58^{d}	32.77 ± 0.99^{d}	0.56 ± 0.02	-7.06 ± 1.90^{d}
T5	120	72.95 ± 0.35^{a}	0.46 ± 0.01	-9.51 ± 0.08^{d}	31.72 ± 0.34^{d}	0.59 ± 0.03	-12.55 ± 1.42

*Statistical differences compared at T1: ^{a}p=0.0433; ^{b}p=0.0072; ^{c}p=0.012; ^{d}p<0.0001; ^{e}p=0.011

Table 3 Mean droplet size (nm), polydispersity index (PDI), and zeta potential (mV) of the P20 and P80 nanoemulsions prepared with *C. longa* essential oil after 0, 1, 7, and 15 days of preparation

Day	Polysorbate 20 (A)			Polysorbate 80 (B)		
	Z-Average (d.nm)	Polydispersity index	Zeta potential (mV)	Z-Average (d.nm)	Polydispersity index	Zeta potential (mV)
D0	72.95 ± 0.35	0.46 ± 0.01	-9.51 ± 0.08	31.72 ± 0.34	0.59 ± 0.03	-12.55 ± 1.42
D1	80.72 ± 2.02^{b}	0.29 ± 0.00^{b}	-6.92 ± 1.65	19.76 ± 0.02^{d}	0.58 ± 0.01	-11.74 ± 1.516
D7	71.02 ± 1.14	0.29 ± 0.03^{a}	-13.58 ± 1.59^{C}	28.70 ± 6.87	0.63 ± 0.18	-25.87 ± 3.53^{g}
D15	60.98 ± 0.66 ^{a}	0.27 + 0.01^{a}	-6.17 + 1.84	12.04 ± 0.03^{e}	0.18 ± 0.02^{f}	13.01 ± 2.92

*Statistical differences compared at D0: ^{a}p<0.0001; ^{b}p=0.0002; ^{c}p=0.0235; ^{d}p=0.0071; ^{e}p=0.0003; ^{f}p=0.0015; ^{g}p=0.0005

Table 4 Mean droplet size (nm) and polydispersity index (PDII) of the P20 and P80 nanoemulsions prepared with *C. longa* essential oil at different temperatures (25 to 60 °C)

Temperature (°C)	Polysorbate 20		Polysorbate 80	
	Z-Average (d.nm)	Polydispersity index	Z-Average (d.nm)	Polydispersity index
25	115.9±0.7	0.21±0.01	11.75±0.04	0.23±0.01
30	114.3±0.6	0.21±0.01	11.44±0.13	0.19±0.02
35	111.5±0.3	0.20±0.01	11.67±0.07	0.18±0.01
40	111.5±0.3	0.22±0.01	11.83±0.05	0.16±0.01
45	113.5±2.3	0.21±0.01	12.16±0.05	0.16±0.01
50	130.2±4.7[a]	0.21±0.01	17.54±5.48[c]	0.34±0.15
55	178.2±11.1[b]	0.17±0.00	70.61±1.78[a]	0.30±0.01
60	324.6±33.6[a]	0.25±0.30	92.16±2.20[a]	0.22±0.02

*Statistical differences compared with 25 °C: [a]$p<0.001$; [b]$p=0.006$; [c]$p=0.0031$

transparent over time. This behavior may reflect a later, yet more efficient, interfacial reorganization, possibly driven by its higher compatibility with *C. longa* essential oil. This affinity may favor the formation of a more cohesive and flexible interfacial film, resulting in lower free energy in the system, which could explain the gradual transition from a translucent to a transparent appearance when migrating to an energetically stable system [35, 36].

With respect to thermal stress, the changes observed in the nanoformulations highlight the critical role of surfactants in the colloidal stability of nanoemulsified systems. According to Mushtaq et al. [37], nonionic surfactants such as P20 and P80 undergo dehydration of their polyoxyethylene head groups at elevated temperatures, reducing their hydrophilicity. This behavior alters the spontaneous

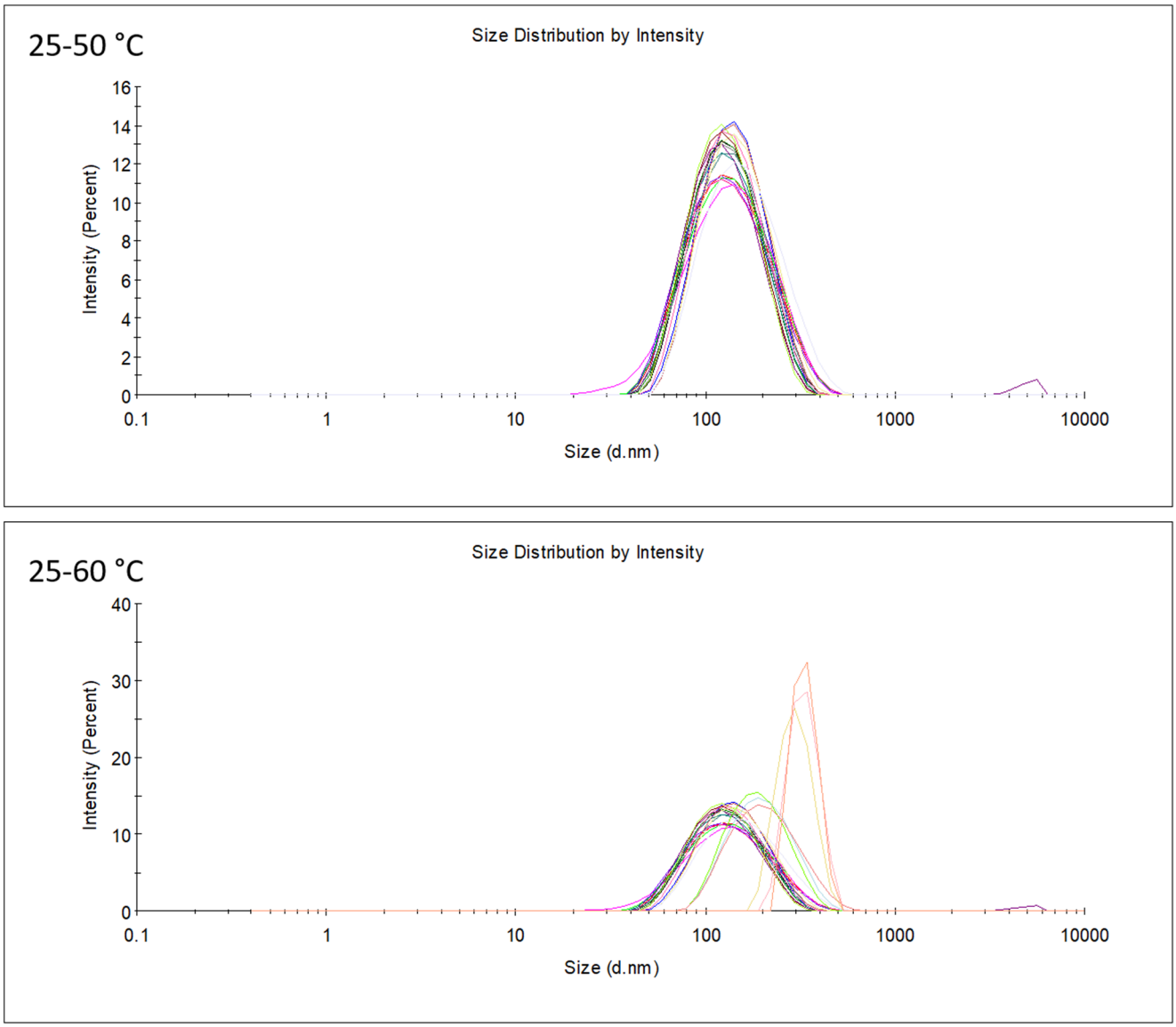


Fig. 6 Thermal stress stability of the polysorbate 20 nanoemulsion at 25–60 °C

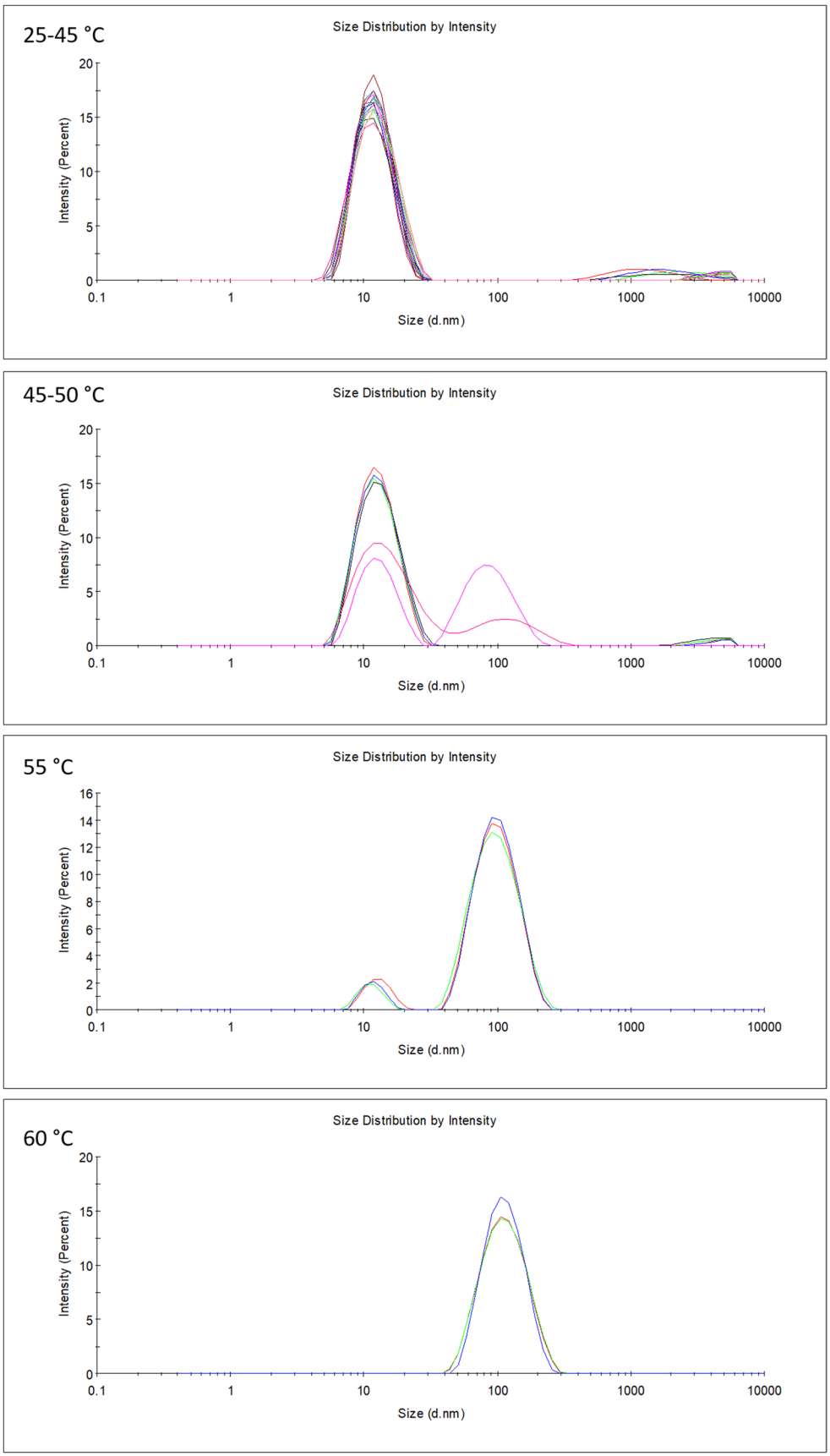


Fig. 7 Thermal stress stability of the polysorbate 80 nanoemulsion at (**A**) 25–45 °C, (**B**) 45–50 °C, (**C**) 55 °C, and (**D**) 60 °C

Table 5 *Curcuma longa* L. nanoemulsion lethal concentration in *Biomphalaria glabrata* juveniles (3–5 and 6–8 mm) and adult (10–12 mm) mollusks after 24 h of exposure

Nanoemulsions lethal concentration in Biomphalaria glabrata					
	LC_{50} (µg/mL)	LC_{90} (µg/mL)	χ^2	df	*p* value
P20					
3–5 mm	19.69 (13.86–26.01)	33.86 (25.70-63.95)	2.2242	5	0.8173
6–8 mm	53.65 (38.18–69.58)	126.42 (92.27-257.45)	3.2959	5	0.6545
10–12 mm	68.44 (53.12–81.89)	115.09 (93.52-195.54)	2.4497	3	0.4845
P80					
3–5 mm	22.39 (15.23–29.70)	44.84 (33.45–76.56)	3.2493	5	0.6770
6–8 mm	28.90 (18.25–39.76)	77.87 (54.47-160.49)	2.3465	4	0.6723
10–12 mm	52.14 (39.30-63.19)	89.72 (72.84-137.38)	1.2679	4	0.8668

*P20: polysorbate 20; P80, polysorbate 80

curvature of the interface, resulting in increased droplet size and polydispersity.

The P20 formulation demonstrated stability up to approximately 50 °C, above which there was a significant increase in the mean droplet diameter and a change in the macroscopic appearance, indicating a collapse of the stabilizing forces. This behavior may be associated with dehydration of the hydrophilic head of the surfactants, increased interfacial fluidity, and progressive instability of the colloidal aggregates, resulting in coalescence and macroemulsion formation [38]. Furthermore, the increase in thermal kinetics intensifies Brownian motion, favoring collisions between droplets and increasing the coalescence rate, especially when the steric barrier becomes insufficient. These observations are in line with studies that relate the molecular structure of surfactants to the robustness of the interface under severe thermal conditions [39].

However, P80 exhibited a micellar profile up to 45 °C, followed by gradual transitions in size distribution and the emergence of a second population at 50 °C, culminating in the predominance of larger structures. The visual transition from a clear liquid to a translucent dispersion further supports this structural reorganization, indicating that despite maintaining a monomodal profile at the end of the heating ramp, the system underwent a significant transformation in its colloidal structure, migrating from a microemulsion to a nanoemulsion [35]. Thus, we can observe that, comparatively, both formulations exhibit a loss of thermal stability from temperatures above 45–50 °C but with distinct behaviors in terms of resistance and transition mechanisms. While the formulation with P80 showed greater thermal robustness and a more progressive transition, the formulation with P20 presented a more abrupt decrease in stability, suggesting that the molecular structure of the surfactant plays a determining role in the response of the nanoemulsions to thermal stress.

Other studies have evaluated the molluscicidal potential of *Curcuma longa* derivatives. However, only a few of these have been developed into formulated products. Silva Filho et al. [40] described the molluscicidal potential associated with *C. longa* essential oil to control *Biomphalaria glabrata* mollusks (8 to 12 mm), which showed a promising LC_{50} of 46.73 µg/mL [40]. The LC50 of the *C longa* nanoemulsion obtained in the actual study ranged from 19.69 to 68.44 µg/mL and varied according to the shell diameter of the mollusk. Juvenile (3 to 5 mm) mollusks appeared more susceptible to low LC_{50} values (19.69 µg/mL for P20 and 22.39 µg/mL for P80) in both *C. longa* nanoemulsions. This is likely because the juvenile organism is not physiologically or metabolically developed, making it more susceptible to nanoemulsion effects.

Among the identified compounds, ar-turmerone stands out among the compounds widely described in the literature regarding its biological activities. Previous studies

Table 6 Mortality of the *Curcuma longa* L. nanoemulsion with polysorbate 20 (P20) and polysorbate 80 (P80) in *Biomphalaria glabrata* mollusks after 24 h

Nanoemulsions mortality (%) in Biomphalaria glabrata after 24 h						
	P20			P80		
µg/mL	3–5 mm	6–8 mm	10–12 mm	3–5 mm	6–8 mm	10–12 mm
Negative control	0±0	0±0	0±0	0±0	0±0	0±0
P20 blank	0±0	0±0	0±0	0±0	0±0	0±0
P80 blank	0±0	0±0	0±0	0±0	0±0	0±0
Positive control	100±0	100±0	100±0	100±0	100±0	100±0
120	100±0	88.8±23.6	88.8±19.24	100±0	100±0	100±0
100	100±0	66.6±47.14	77.7±38.5	100±0	88.8±19.2	88.8±19.2
80	100±0	77.7±0	66.6±33.3	100±0	88.8±19.2	88.8±19.2
60	100±0	66.6±47.14	55.5±19.2	88.8±19.2	88.8±19.2	55.5±19.2
40	88.8±19.2	44.4±0	0±0	88.8±19.2	66.6±33.3	33.3±33.3
20	66.6±33.3	0±0	0±0	55.5±19.2	44.4±19.24	0±0
10	0±0	0±0	0±0	0±0	0±0	0±0

*P20: polysorbate 20; P80, polysorbate 80

have reported its insecticidal activity against different species of insects, highlighting its potential as a natural agent for agricultural control. However, although there is evidence regarding its insecticidal activity, studies specifically describing the molluscicidal activity of this sesquiterpene are still scarce. Thus, the presence of ar-turmerone in the evaluated essential oil suggests a possible contribution to the molluskicidal activity observed in this work [41, 42].

For an essential oil to be considered a promising molluscicidal, it must have an LC90 < 100 µg/mL, as established by the World Health Organization [43]. In this context, both nanoemulsions (P20 and P80) can be considered promising agents for the control of schistosomiasis. Essential oils have been described as potential alternatives to synthetic molluskicidal oils because of their volatility and biodegradability characteristics, which can reduce their environmental persistence and bioaccumulation potential. Thus, compared with conventional pesticides, *Curcuma longa* essential oil may represent a potentially sustainable alternative. Studies conducted with the nontarget organism *Artemia salina* demonstrated a median lethal concentration (LC50) of the essential oil of 319.82 µg/mL, which is approximately five times greater than the LC_{50} observed for *Biomphalaria glabrata* in the present study, suggesting some biological selectivity. Furthermore, *C. longa* has a history of traditional use as a condiment and, more recently, as a nutraceutical associated with different bioactivities and protective effects. However, further studies involving other nontarget organisms, different trophic levels, and environmental scenarios are still needed to confirm its environmental safety and its potential as an alternative to conventional molluscicidal.

In this way, the development of molluscicidal for the control of schistosomiasis with *Curcuma longa* is a tangible reality, since there are cultivars worldwide, relatively simple technologies for obtaining nanoemulsions, selectivity, and biorational use, as well as the possibility of using *Curcuma longa* to control schistosomiasis.

5 Conclusion

The essential oils of *C. longa* rhizomes include major constituents of ar-turmerone, turmerone, and curlone. The nanoemulsions formulated with *C. longa* essential oil using polysorbate 20 and polysorbate 80 demonstrated stability under thermal stress, with polysorbate 20 maintaining stability between 25 and 50 °C and polysorbate 80 remaining stable up to 60 °C. Both formulations also exhibited significant molluscicidal activity against *Biomphalaria glabrata* at different developmental stages, reinforcing their potential as sustainable agents for schistosomiasis control. In particular, compared with polysorbate 20, polysorbate 80 showed greater efficacy, with lethal concentration values (LC_{90}) against adult mollusks (10–12 mm) of 89.72 µg/mL and 115.09 µg/mL, respectively. These findings highlight the decisive role of surfactant selection, as well as the interactions between oil constituents and micellar systems, in determining droplet stability, size distribution, and overall performance of the formulations.

Author Contributions KNFG, FPM - wrote the main manuscript text. DSS, KCP, - prepared figuresKNFG - realized the biological assays. FMP, DSS, and JN - prepared the nanoformulations.FMP, DSS, KCP, and JN- realized the physico-chemical assays. CPF and LR - wrote and revised the manuscript.RXF - revised the manuscript.All authors reviewed and approved the submitted version of the manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics Declaration Not applicable.

Competing interests The authors declare no competing interests.

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