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Research Article

Eugenia supra-axillaris Essential Oil and Its Nanoemulsion: Chemical Characterization, *In Vivo* Anti-Inflammatory, Analgesic, and Antipyretic Activities

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The use of standard synthetic medications to treat inflammatory illnesses is associated with several negative effects. It has been shown that medicinal plants and their by-products are useful for safely treating inflammation. Herein, the essential oil of *Eugenia supra-axillaris* (family: Myrtaceae, ESA-EO) was isolated and further chemically characterized by GC-MS, and then, its nanoemulsion (ESA-EO-NE) was prepared. In addition, the anti-inflammation against the carrageenan-induced rats, the analgesic, and antipyretic activities of ESA-EO and ESA-EO-NE were evaluated in rats. Forty-three compounds were identified via GC-MS and categorized as mono- (61.38%) and sesquiterpenes (34.86%). d-limonene (32.82%), α-pinene (24.33%), germacrene-D (4.88%), α-humulene (4.73%), α-cadinol (3.39%), and *trans*-caryophyllene (3.15%) represented the main components. The administration of ES-EO and ES-EO-NE (50 and 100 mg/kg) demonstrated strong, dose-dependent inflammation inhibition capabilities in the model of rat paw edema, in comparison with both the reference drug and control. Reduced levels of malondialdehyde (MDA), increased levels of glutathione (GSH), and decreased levels of the proinflammatory cytokines (TNF-α), nitrosative (NO), and prostaglandin E2 (PGE2) in paw tissues all contributed to these substantial reductions in inflammation. Moreover, the oral administration of ESA-EO and ESA-EO-NE (50 and 100 mg/kg) exhibited potent analgesic and antipyretic activities in rats. Although the higher dose of ESA-EO and ESA-EO-NE (100 mg/kg) displayed delayed anti-inflammatory activity, they have long-lasting inflammation inhibition with fast onset and long-standing analgesic effects better than reference drugs. Furthermore, the most effective antipyretic efficacy was provided by ESA-EO-NE (100 mg/kg). These results provide insight into the possible therapeutic application of ESA-EO and its nanoemulsion against various inflammatory and painful illnesses as well as hyperthermia ailments.

1. Introduction

Inflammations are responses to different body infections, including loss, damage, and/or irritation of the cells [1]. Several associated symptoms with chronic inflammations are observed, including pains and fevers [2, 3]. Recently, the incidence of inflammatory diseases has been raised [1]. Synthetic drugs for inflammation management, such as nonsteroidal anti-inflammation drugs (NSAIDs), demonstrated symptom relief but with serious consequences and side effects that endangered life [4, 5]. Since ancient times, the natural by-products from medicinal plants and their secondary metabolites have been used to treat several inflammatory disorders due to their safety compared with synthetic drugs [6, 7].

Family Myrtaceae includes more than 130 genera with around 3000 plant species and is widely distributed worldwide, especially in warm areas. The Myrtaceae plants are dicotyledonous, evergreen, and woody. Most of the plants of this family are significant rich resources of essential oils (EOs) [8]. The *Eugenia* genus is the biggest one of the genera of this family and is very common and distributed in the tropical regions of Australia and America [9]. Numerous traditional uses were documented for the *Eugenia* plants, such as a liver tonic as well as treatment of diabetes, wound healing, fever, head ringworm infections, and diarrheal in addition to their nutritional values [9]. Different extracts and EOs derived from *Eugenia* plants were described to have many biological potentialities, including antimicrobial, antiviral, antigenotoxic, anti-inflammation, antiulcer, cardio-, hepato-, chemo-, and radioprotective, antiallergic, antitumor, antidiabetic, and antidiarrheal, and hypoglycemic activities [8, 9]. Several phytoconstituents were also characterized from *Eugenia* plants, including phenolic acids, flavonoids, sterols, terpenes, EOs, and other phytochemicals [8–11].

E. supra-axillaris Spring ex Mart is a commonly cultivated plant in subtropical and tropical areas. Chemically, several phenolic metabolites were characterized from the different extracts of this plant, including phenolic acids and flavonoids [12]. Some reported data revealed the significant roles of the extract of *E. supra-axillaris* as hepatoprotection [13], free radical scavenging, anti-inflammation, pain killing, and antipyretic potentialities [14]. The previous chemical and biological studies of the *E. supra-axillaris* leaf and fruits EOs exhibited that these oils are rich with mono- and sesquiterpenes with significant antimicrobial, anticancer, and antiviral effects [8, 15].

The loading of EOs into nanosystems has become the most recent significant strategy for enhancing the physicochemical properties including the stability, volatility, and toxicity and thus enhancing the active gradients stability and penetration in the cellular uptake and tissues [16–18].

The pmain objectives of the present work are as follows: (i) chemical profiling of EO derived from the shoots of *E. supra-axillaris* cultivated in the Nile delta of Egypt; (ii) comparison between the EO of the shoots in the present study and previous studies' essential oil of the leaf and fruits using chemometric analysis, including principal components analysis (PCA) and agglomerative hierarchical clustering (AHC); (iii) preparation of the nanoemulsion for this EO (ESA-EO-NE); and (iv) evaluation of analgesic, antipyretic, and anti-inflammatory of the EO and ESA-EO-NE in rats.

2. Materials and Methods

2.1. Plant Parts and Collection. *E. supra-axillaris* shoots were collected in September 2020 from the trees cultivated in the El-Orman Botanical Garden in Giza (30 01′45″ N 31 12′47″ E), Egypt. The collection and authentication were performed via the senior botanist, Prof. Mohamed A. Gibali. The plant material was catalogued under voucher number (Y190-ESA-93 390z) and kept in the herbarium of the El-Orman Botanical Garden in Giza, Egypt. All the collected fresh shoots were directly crushed into very small pieces and then subjected to hydrodistillation.

2.2. EO Isolation by Hydrodistillation. The *E. supra-axillaris*' EO (ESA-EO) was isolated via hydrodistillation of the fresh shoot's pieces (200 g) by the Clevenger apparatus for three hours. Then, EO was decanted, separated, and dried via anhydrous sodium sulphate (0.5 g). Three EO samples were obtained from three separated plant samples through the same above sequence.

2.3. GC-MS Analysis of EO. The previously described technique was followed for each EO sample's analysis using gas chromatography-mass spectrometry (GC-MS) [17–19]. The employed equipment is a TRACE GC Ultra Gas Chromatograph from Thermo Fisher Scientific, Waltham, Massachusetts, with an ISQTM Single Quadrupole Mass Spectrometer serving as the instrument's detector. In the GC-MS system, the TR-5 MS column (30 m × 0.32 mm inner diameter, 0.25 μm film thickness) was installed. Till 240°C was attained after rising from 60°C at a rate of 4.0°C per minute for 1 minute, and helium was used as a carrier gas with an average flow rate of 1.0 mL·min^{-1} and a split ratio of 1 : 10. At 210°C, the injector and detector were kept. Dilutions of the mixes (1 : 10 hexane, v/v) in 1 μL were used to make the injections. Mass spectra were generated employing a spectral range of m/z 40–450 and electron ionisation (EI) at 70 eV.

2.4. GC-Flame Ionization Detector (GC-FID) Analysis. The HP-5890 series II equipment (Hewlett-Packard, Palo Alto, CA, USA), which is equipped with two silica capillary columns (30 m 0.25 mm; film thickness: 0.25 μm), an HP-Wax, and a DB-5 (Agilent, Santa Clara, CA, USA), was used to conduct the GC analysis. With a splitless injection, carrier gas N_2 flowing at a rate of 2 mL/min, and injector and detector temperatures set to 250°C, the oven was programmed to heat up from 60°C to 220°C at a rate of 5°C per minute. After GC-MS analysis, all the EO samples were collected in one glass vial and saved at 4°C for biological estimations.

2.5. EO Characterization. Depending upon the database of NIST, collection of the Wiley spectra, along with the software of automated mass spectral deconvolution and identification (AMDIS), and retention indices of n-alkanes (C8–C22). EO components' identification was performed. The identification was supported by comparing the retention times with the pure authentics via the retention indices' linear in relationship with the *n*-hydrocarbons series, matching computer programmes with commercial[32,33], and created mass spectra from a library comprised of pure compounds, knew oil components, and information from MS literature.

2.6. Chemometric Studies of Eugenia supra-axillaris Different Parts Using the Main Essential Oil Components. Further chemometric analysis of the *E. supra-axillaris* different parts was performed via the major compounds of the EO [8]. The established principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were performed using a matrix of the EOs of shoots (present study), leaves, and fruits [8] with 16 main compounds (relative concentration >2%) by version 2018-XLSTAT statistical software (Addinsoft Inc., New York, USA).

2.7. Preparation of ESA-EO-NE. Tween 80 was used to create ESA-EO-NE as a nonionic surfactant. Tween 80 was incorporated into the organic phase (1 : 1 w/w) of EO. At 25°C and vigorous stirring, the organic phase mixture was steadily added to the distilled water (aqueous phase). The formed emulsion had been sonicated for about fifteen minutes at 20°C with an output power of 750 W using an ultrasonic device (Sonics & Materials, Inc., 53 Church Hill Rd., Newtown, CT, USA) with a 13 mm probe diameter [20].

2.8. Zeta Potential and Droplet Size Characterization. The average size, size distribution, and zeta potential were calculated at 23°C using a dynamic light scattering (DLS) apparatus (PSS, Santa Barbara, CA, USA) utilizing the 632.8 nm line of a He Ne laser as the incident light with an angle of 90° and the zeta potential with an external angle of 18.9° [21].

2.9. Biological Assessments Experimental Section

2.9.1. Drugs and Chemicals. Carrageenan, celecoxib, and diclofenac sodium were acquired from the Sigma-Aldrich (Darmstadt, Germany). From the Angel Yeast Company (Hubei, China), brewer's yeast was obtained. GSH and MDA were purchased from BioDiagnostic in Cairo, Egypt, whereas ELISA kits from Cloud-Clone in Katy, Texas, USA, were used to measure TNF-α. Nitric oxide (NO) levels were additionally determined using chemical methods and spectrophotometry. The highest grade commercially available organic solvents and all of the used compounds were purchased.

2.9.2. Experimental Animals. Female adult Wistar rats weighing 140–170 g were obtained from the animal house of the National Research Centre (NRC) in Cairo, Egypt. The rats were housed in metal cages inside the NRC animal house under completely professional conditions, with access to food and water. All experimental procedures and treatments were carried out in accordance with the US National Institute of Health policies and through the NRC Committee, Egypt's ethical policy (ethical approval ID: CU/F/16/21).

2.9.3. Acute Toxicity Assessment. The acute toxicity of the ESA-EO and ESA-EO-NE was evaluated using 24 female Swiss albino mice that were divided into three separate groups ($n = 8$). Group 1 was orally administrated by ESA-EO at a dose of 2.0 g/kg·b.w. Group 2 was orally administrated by ESA-EO-NE at a dose of 2.0 g/kg·b.w. Group 3 (the control group) received the same amounts of distilled water orally. All of these mice were kept under close monitoring for mortality for 24 hours. Along with their behaviours, all of the mice's bodily changes—including those to their respiratory, circulatory, hair, and skin—were noticed [22, 23].

2.9.4. Analgesic, Antipyretic, and Anti-Inflammatory Potentialities of ESA-EO and ESA-EO-NE

(1) Carrageenan-Induced Rat Paw Edema. Randomly, forty-two adults female Wistar rats (weights: 140–170 g body weight, the pregnant rats were excluded) were divided into eight groups (6 rats each). Group I was kept as a negative control (1 ml saline per os) and injected with 0.1 ml saline at the subplantar region of the left hind paw. The induction of inflammation was carried out by subplantar injection of freshly prepared 1% solution of carrageenan in saline (0.9%, 0.1 ml per rat) into the subplantar region of the right hind paw of the seven last groups as previously described [24]. Group II served as the positive control and was only given 1 ml of saline per os. Group III was orally given diclofenac at a dose of 30 mg/kg (the standard drug); Group IV was orally given celecoxib at a dose of 50 mg/kg (the standard drug); Group V was given ESA-EO in two dose levels (50 and 100 mg/kg); and Groups VI and VII were given ESA-EO-NE in two dose levels (50 and 100 mg/kg). All treatments were given thirty minutes before subplantar injections of carrageenan. The MNT-150 vernier calliper (Jiangsu Goldmoon Industry Co., Ltd., Shanghai, China) was employed for measuring the thickness of the paw (in mm) after series duration periods (1, 2, 3, and 24 h) and compared it to the original hind paw thickness of each rat to determine the thickness of the edema. Equations (1) and (2) were used to determine the reduction of edema thicknesses at the specified intervals and to express the inflammatory-fighting action as a percentage of edema change.

$$\text{Edema}'\text{change}\% = \frac{V_t - V_0}{V_0} \times 100, \quad (1)$$

where V_0 and V_t are the thicknesses (mL) before and after (by t hour) injection of carrageenan, respectively.

$$\text{Edema}' \text{inhibition}\% = \frac{E_c - E_t}{E_c} \times 100, \quad (2)$$

where E_c is control and treated rats' edema volumes, respectively.

After an overnight fast, all of the rats were sacrificed through a cervical dislocation while being put under anaesthesia with a large dosage of ketamine. Their examined hind right paws were then severed at the level of the calcareous bone. After the paws were degloved to eliminate the bones, they were instantly frozen in liquid nitrogen and stored until needed [25]. The potency of the treated groups by ESA-EO and ESA-EO-NE was expressed as described in the following equation [24, 26]:

$$\%\,\text{Potency} = \frac{\%\ \text{inhibition of edema of the treated (at 3 h effect)}}{\%\ \text{inhibition of edema of the reference drug (at 3 h effect)}} \times 100. \quad (3)$$

(2) Analgesic Potentiality. The *in vivo* standard hot plate experiment was performed using the standard hot plate technique. Each group had six 20–25 g albino mice of either sex. Mice were placed on a hot plate that was kept at 52 (±1)°C (7280 Ugo Basile Biological Research Apparatus Company, Comerio, Italy) for 30 minutes to be completely examined. The equipment mentioned previously [25]. It comprises of a hot plate, which was used to test the rat. It comprises of a foot switch-operated timer, a metal hot plate surface with a 20 cm diameter adjusted to 51–53°C, and a plexiglass cage that fits the hot metal surface [10]. Prior to the experiment, rats were trained to tolerate the hot plate by having them spend 15 minutes each day resting on a plate that was kept at room temperature. Before testing, all groups received the vehicle and/or the various treatment doses as described above for 60 min. The test was then conducted by carefully putting each animal onto a 52°C hot plate. After the application, the latency to display nociceptive behaviours such as licking paws or leaping off the hot plate was measured at 30, 60, 90, and 120 minutes. In order to avoid damage to tissues after administering the test substances or the saline, a maximum cut-off time of 15 seconds was set [11]. After the application, response times were timed at 30, 60, 90, and 120 minutes. Equation (4) was used to calculate the percentage of protection [26].

$$\text{Protection}\% = \frac{T_1 - T_0}{T_0} \times 100, \quad (4)$$

where T_0 and T_1 are the time mean' latencies of the control and tested samples, respectively.

(3) Antipyretic Effect. The female Wistar rats (42 rats) were divided into seven groups (6 rats/group). A digital thermometer was used to take the rectal temperature before administering the yeast. The following stage involved injecting each animal with a pyrogenic dosage of Brewer's yeast (1 ml/100 g bwt of 44% yeast suspended in saline). The temperature was taken 24 hours after the yeast injection to compare the antipyretic effect to the baseline line of elevated body temperature. The experiment was carried out on pyretic rats, defined as those with a rectal temperature increase of higher than 0.3°C. Rectal temperature was measured after 30, 60, and 120 minutes of intervention with a single oral administration of the ESA-EO and ESA-EO-NE with the two doses (50 and 100 mg/kg), celecoxib and diclofenac as two reference drugs (standard drugs), or saline (control) [18].

(4) Homogenate Preparation. Tissues were thoroughly cleaned before being rinsed in the ice. They were carefully blotted between the filter paper folds and weighed using an analytical balance. At 40°C in 0.05 M phosphate buffer (pH 7), 10% of the homogenate was prepared using a polytron homogenizer. To remove the nuclei, erythrocytes, unbroken cells, cell debris, and mitochondria from the homogenate, it was centrifuged at 10,000 rpm for 20 min. The tissue's composition of proteins was ascertained using the Bradford method [27] utilizing the protein estimation kit from Genei, Bangalore. The cytoplasmic extract, or supernatant, was used in accordance with manual instructions to measure the amounts of TNF-α protein. The ELISA kit was measured with an ELISA reader. A plate reader (Stat Fax 2200, Awareness Technologies, Florida, USA) was employed to detect colour absorbance for the enzyme-linked immunosorbent assay (ELISA) at an OD range of 450 nm.

(5) Assessment of MDA, GSH, Inflammatory Cytokines, and Nitric Oxide (NO). The Biodiagnostic, Egypt, kit materials were used to colorimetrically measure MDA and GSH. Utilizing specific ELISA kits and following the manufacturer's instructions, TNF-α was quantified. In accordance with Miranda et al. [28], the content of NO has been determined. As only a stable by-product of the NO autoxidation, vanadium (III) converts nitrates to nitrites and/or nitric oxide, both of which are caught by Griess reagents (premixed 50 μL sulfanilamide (2%) in HCl (5%) and *N*-(1-naphthyl) ethylenediamine dihydrochloride (0.1%, 50 μL)). Spectrophotometry can be used to detect the pink metabolite that results from the Griess reaction at 540 nm.

(6) PGE2 Gene Expression Analysis. With Direct-zol RNA MiniPrep Plus (Cat# R2072, ZYMO RESEARCH CORP., USA), the isolation of the total RNA was carried out from the homogenised tissues from the various groups, and its quantity and quality were assessed by a Beckman dual spectrophotometer (USA). For reverse transcription and PCR of the extracted RNA, the SuperScript IV One-Step RT-PCR kit (Cat# 12594100, Thermo Fisher Scientific,

Waltham, Massachusetts, USA) was used. On StepOne apparatus (Applied Biosystem, USA), the thermal profiling was performed using a 48-well plate as follows: it takes 10 min. to complete reverse transcription at 45°C, 2 min. to complete RT inactivation at 98°C, and 40 cycles of 10 sec. at 98°C, 10 sec. at 55°C, and 30 seconds at 72°C to complete the initial denaturation phase. Following the RT-PCR run, the data were represented as cycle threshold (Ct) for the target genes and housekeeping genes. Applying the average critical threshold (CT) expression values of the housekeeping gene β-actin, P21 and P16 were normalised for variation in each target gene's expression. The relative quantitation (RQ) of each target gene is calculated by the $\Delta\Delta$Ct technique. The sequences of the primer for the genes housekeeping, β-actin and PGE2, were previously provided [18].

2.10. Statistical Study. The mean and standard deviation (SD) were used for expressing each biological result. The statistical analysis was constructed by utilisation of one-way analysis of variance (ANOVA), the Tukey test for confirmation, and multiple comparisons. The threshold for statistical significance was set at P 0.05. The statistical analysis was carried out using GraphPad Prism (Version 7.00).

3. Results and Discussion

3.1. Chemical Profile of ESA-EO. The pale yellow ESA-EO (2.85% v/w) with a sharp odor was isolated via hydrodistillation of the fresh shoots. This yield was found very near from the earlier reported yielding of the leaves and fruits of Egyptian-cultivated *E. supra-axillaris* [8]. From GC-MS analysis as presented in Figure 1, forty-three compounds were assigned and listed in Table 1 along with their retention times (Rt), relative concentrations (Rel. Conc. %), and Kovats retention indexes (KI). All the identified constituents were found to belong to terpenes, especially the mono- and sesquiterpenes.

The preponderance of the terpenes, especially mono- and sesquiterpenes, in our findings, was in complete harmony with the documented data from the different *Eugenia* plants such as *E. egensis*, *E. flavescens*, *E. patrisii*, *E. polystachya* [29], *E. aromatic* [30], and *E. caryophyllata* [31], in addition to the leaves and fruits of *E. supra-axillaris* [8, 15]. More specifically, the presence of the mono- and sesquiterpenes only in the EO of this plant completely agrees with the published data of Aboutabl and his teamwork [8].

Monoterpenes were characterized as abundant constituents with a relative concentration of 61.38% including hydrocarbons as the main (59.74%), and traces of the oxygenated forms (1.91%). Eight compounds were identified as overall monoterpene hydrocarbons comprising of d-limonene (32.82%) and α-pinene (24.33%) as the major and *trans*-β-ocimene (0.13%) as the minor one. Otherwise, nine compounds as minors were identified as overall oxygenated monoterpenes in which α-terpineol is a major one (0.58%). The abundance of monoterpenes was already common in EOs derived from some *Eugenia* species such as *E. aromatic* [30], and leaves and fruits of *E. supra-axillaris* [8, 15]. Also, the high relative concentrations of d-limonene and α-pinene in our results agreed with the reported data of EOs of *E. aromatic* [30] and *E. supra-axillaris* leaves and fruits [8, 15].

The sesquiterpenes were found as the second component with a relative concentration of 34.86%, involving sesquiterpene hydrocarbons (21.4%) and oxygenated compounds (13.47%). Four-teen sesquiterpene hydrocarbons were identified in which germacrene-D (4.88%), α-humulene (4.73%), and *trans*-caryophyllene (3.15%) represented the main components while α-copaene (0.07%) is a minor. As well, twenty oxygenated sesquiterpenes were assigned, encompassing α-cadinol (3.39%) and *t*-muurolol (1.88%) as majors and humulene oxide (0.12%) as a minor compound. The majority of the sesquiterpenes harmonized with the documented EO components of *E. egensis*, *E. flavescens*, *E. patrisii*, *E. polystachya* [29], *E. aromatic* [30], and *E. caryophyllata* [31] and *E. supra-axillaris* leaves and fruits [8, 15]. Some published data described that caryophyllene and cadinol are major components in *E. supra-axillaris* leaf EO and *E. caryophyllata* [31] but minors and/or absent in *E. supra-axillaris* fruits EO [8].

3.2. Chemometric Analysis Results. Based on the main components of the EO of *E. supra-axillaris* shoots (present study), leaves, and fruits, the results of the further cluster assessment are presented in Figure 2. The PCA results revealed that EOs derived from the shoots and leaves are closely related to each other via the abundance of the two monoterpene hydrocarbons, α-pinene and d-limonene. Otherwise, the EO afforded from the fruits clearly showed variations than shoots and leaves, by the presence of eugenol and methyl eugenol [8, 9, 32–34]. The AHC results presented in Figure 2 exhibited a significant correlation between the shoots and leaves with $r = +52.15$. Otherwise, the fruits did not exhibit any correlation with the shoots and leaves with a negative similarity (−27.85). These results were found by the abundance of myrcene, 1,8-cineole, eugenol, and methyl eugenol in the EO derived from the fruits of this plan although these compounds were found as minors and/or absent in the leaves and shoots [8, 9]. All these analyses revealed that there is a similarity in the chemical composition of EOs afforded from the leaves and shoots along with significant differences with the fruit EO that might be ascribed by the variation of the plant part. The effects of variation of the plant part on the EOs chemical compositions were previously described [32–34].

3.3. ESA-EO-NE Characterization. ESA-EO has been nanoemulsified and can enhance the physicochemical properties while overcoming the drawbacks of pure oil, such as its low aqueous phase solubility, high volatility, and limited long-term stability. Nanoemulsions have unique characteristics like higher physicochemical stability and contact surface area because of their nanometric dimension [35]. High-energy ultrasonic emulsification techniques were used to create nanoscale ESA emulsion and achieve droplet sizes <100 nm. Deionized water was combined with the

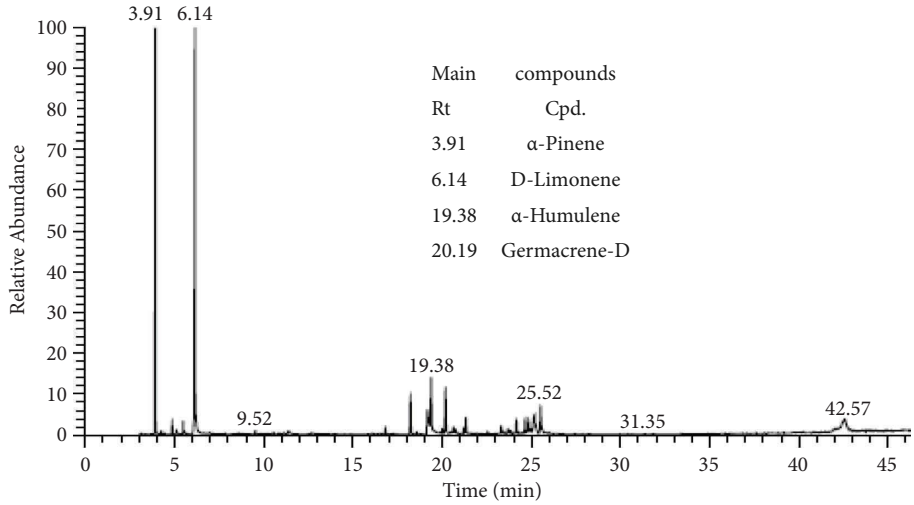

Figure 1: GC-MS chromatogram of EO of *Eugenia supra-axillaris* shoots.

Table 1: Essential oil constituents of the *Eugenia supra-axillaris* shoots.

No	Rt	Rel. Conc. %[a]	Compound name	KI		Type	Identification
				Lit	Cal		
1	3.91	24.33 ± 1.12	*α*-Pinene	931	929	MH	KI, MS
2	4.26	0.18 ± 0.01	Camphene	953	955	MH	KI, MS
3	4.86	1.61 ± 0.03	*β*-Pinene	964	965	MH	KI, MS
4	5.09	0.38 ± 0.04	*α*-Myrcene	988	990	MH	KI, MS
5	5.55	0.15 ± 0.01	3-Carene	1011	1013	MH	KI, MS
6	6.14	32.82 ± 2.05	d-Limonene	1022	1024	MH	KI, MS
7	6.64	0.13 ± 0.01	*trans*-*β*-Ocimene	1050	1049	MH	KI, MS
8	6.94	0.14 ± 0.01	*γ*-Terpinene	1057	1055	MH	KI, MS
9	8.28	0.06 ± 0.00	Linalool	1094	1096	OM	KI, MS
10	8.85	0.08 ± 0.00	d-Fenchyl alcohol	1109	1110	OM	KI, MS
11	9.51	0.34 ± 0.02	*trans*-Pinocarveol	1139	1137	OM	KI, MS
12	9.80	0.23 ± 0.01	Isopulegol	1145	1146	OM	KI, MS
13	10.54	0.18 ± 0.01	Borneol	1149	1149	OM	KI, MS
14	11.11	0.21 ± 0.01	*cis*-p-Mentha-1(7),8-dien-2-ol	1165	1164	OM	KI, MS
15	11.38	0.58 ± 0.03	*α*-Terpineol	1169	1171	OM	KI, MS
16	12.65	0.12 ± 0.01	Carveol	1207	1205	OM	KI, MS
17	16.58	0.11 ± 0.00	*α*-Terpineol acetate	1352	1350	OM	KI, MS
18	16.82	0.54 ± 0.03	*α*-Ylangene	1375	1376	SH	KI, MS
19	17.29	0.07 ± 0.00	*α*-Copaene	1374	1372	SH	KI, MS
20	18.22	3.58 ± 0.11	*α*-Elemene	1388	1487	SH	KI, MS
21	19.06	0.16 ± 0.01	*β*-Cubebene	1389	1391	SH	KI, MS
22	19.17	3.15 ± 0.07	*trans*-Caryophyllene	1406	1405	SH	KI, MS
23	19.38	4.73 ± 0.13	*α*-Humulene	1448	1450	SH	KI, MS
24	20.00	0.48 ± 0.02	*γ*-Muurolene	1480	1476	SH	KI, MS
25	20.19	4.88 ± 0.12	Germacrene-D	1480	1480	SH	KI, MS
26	20.46	0.14 ± 0.01	*β*-Selinene	1482	1483	SH	KI, MS
27	20.62	0.64 ± 0.03	*α*-Muurolene	1491	1490	SH	KI, MS
28	20.75	0.47 ± 0.02	Bicyclogermacrene	1494	1496	SH	KI, MS
29	20.84	0.37 ± 0.01	Eremophilene	1503	1501	SH	KI, MS
30	21.21	0.52 ± 0.02	*γ*-Cadinene	1510	1514	SH	KI, MS
31	21.33	1.66 ± 0.03	*δ*-Cadinene	1519	1523	SH	KI, MS
32	23.30	1.05 ± 0.04	Ledol	1565	1567	OS	KI, MS
33	23.43	0.35 ± 0.01	Globulol	1576	1577	OS	KI, MS
34	23.68	0.61 ± 0.02	Caryophyllene oxide	1583	1583	OS	KI, MS
35	23.98	0.13 ± 0.00	Veridiflorol	1590	1589	OS	KI, MS
36	24.16	1.28 ± 0.06	Calarenepoxide	1592	1594	OS	KI, MS

Table 1: Continued.

No	Rt	Rel. Conc. %[a]	Compound name	KI		Type	Identification
				Lit	Cal		
37	24.49	0.12 ± 0.01	Humulene oxide	1605	1607	OS	KI, MS
38	24.65	1.61 ± 0.07	Fonenol	1627	1625	OS	KI, MS
39	24.84	1.45 ± 0.08	Cubenol	1645	1642	OS	KI, MS
40	25.12	1.35 ± 0.06	*t*-Cadinol	1640	1638	OS	KI, MS
41	25.18	1.88 ± 0.09	*t*-Muurolol	1640	1641	OS	KI, MS
42	25.52	3.39 ± 0.14	*α*-Cadinol	1652	1653	OS	KI, MS
43	25.85	0.25 ± 0.01	Khusinol	1658	1661	OS	KI, MS
		96.51	Total				
		59.74	Monoterpene hydrocarbons (MH)				
		1.91	Oxygenated monoterpenes (OM)				
		21.39	Sesquiterpene hydrocarbon (SH)				
		13.47	Oxygenated sesquiterpene (OS)				

[a]Values are the means of three replications (percentage ± standard deviation). Rt, retention time (min); KI Cal, calculated Kováts retention index on DB-5 column in reference to *n*-alkanes (C8–C22); KI Lit, Kováts retention index from literature.

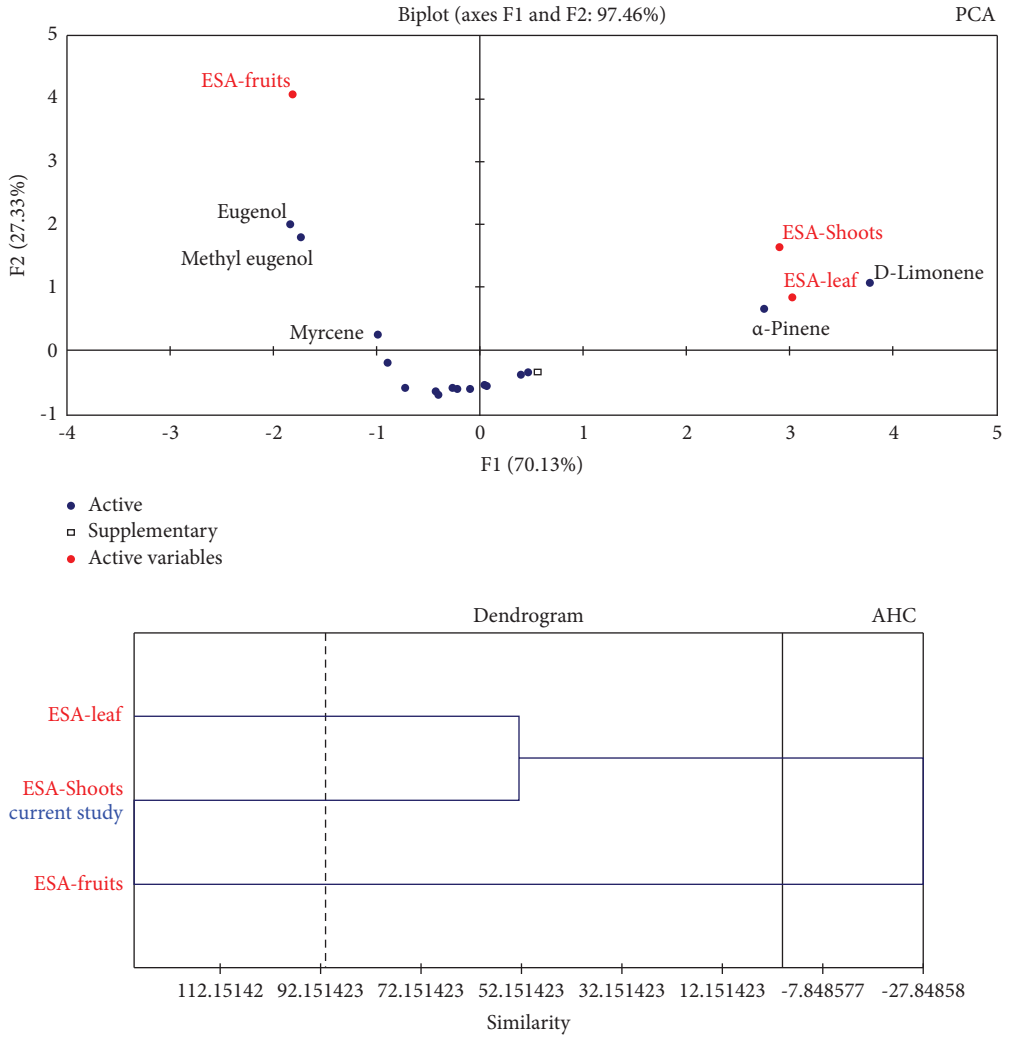

Figure 2: Chemometric studies, principal component analysis (PCA), and agglomerative hierarchical clustering (AHC), of the shoots (current study), leaf, and fruits *Eugenia supra-axillaris* via the main components of the essential oil.

hydrophilic surfactant polysorbate (Tween 80) to create an ESA-EO-NE. The droplet size distribution of the prepared nanoemulsion was determined. The average droplet size diameter was 56.9 ± 0.44 nm, with 25% of the distribution falling below 20.51 nm, 50% < 55.23 nm, 75% < 100.43 nm, 80% < 150.29 nm, and 90% of the distribution less than

200.30 nm (Figure 3(a)). *A. heterophylla* EO nanoemulsion (ESA-EO-NE) had a mean droplet size diameter of 106 ± 0.65 nm, with 25% of the distribution <62.3 nm and 75% < 153.5 nm [17]. Tween 80 concentration and the sonication time both had a significant impact on the creation of the nanoemulsion [21]. When the hydrophile-lipophile balance (HLB) value of the surfactant couple aligns with the HLB value of the oil, a nanoemulsion's small droplet size can be determined [36].

The nanoemulsion's average zeta potential was −1.84 mV (Figure 3(b)). The surface potential of the nanoemulsion droplets and the zeta value are connected. *A. heterophylla* EO nanoemulsion had a zeta potential value of 4.64 mV [17]. Zeta potential value and nanoemulsion formulation stability were associated. When the zeta potential value is greater than or equal to ±30 mV, the maximal stability of the nanoemulsion can be determined [20].

3.4. Results of Anti-Inflammatory Effect

3.4.1. Acute Toxicity Study. The obtained results demonstrated that ESA-EO and its nanoemulsion (ESA-EO-NE) showed no observable signs of toxicity or mortality up to a dose of 2000 mg/kg, thus indicating that the median lethal dose (LD_{50}) of both ESA-EO and ESA-EO-NE could be higher than 2000 mg/kg in mice.

3.4.2. Carrageenan-Induced Rat Paw Edema. Using a carrageenan-induced rat paw edema model, the in vivo anti-inflammatory potency of ESA-EO and its nanoemulsion (ESA-EO-NE) was evaluated [26, 27]. Comparing the pharmacological efficacy to the reference medications celecoxib and diclofenac, the percentage of edema inhibition was used to express the effectiveness (Table S1; Figure 4). The anti-inflammatory activity results showed that only long-lasting anti-inflammatory effects of over 50% at 24 hours were observed in groups that received ESA-EO-NE (100 mg/kg), with edema-inhibitory effects of 53.61%, which were slightly superior to celecoxib's (55.41%) but more potent than short-acting diclofenac's (38.50%). The latter was also still less active than ESA-EO (100 mg/kg) with a mean inhibitory effect (46.67%), while the others did not show any pleased effect.

It is shown that the most potent activity at the 4th h postadministration was gained by all tested candidates with variant degrees. The results showed that the administration of ESA-EO-NE at two different doses (50 and 100 mg/kg) has the best edema inhibitory effect (75.82% and 93.12%, respectively) and is even superior to the reference drug celecoxib (60.64%) but slightly lower in activity than the other reference drug diclofenac (85.50%). It is noted also that the two doses of ESA-EO have optimal anti-inflammatory activity, which may be inferior to diclofenac (85.50%) in the percentage of edema inhibition but is more potent than celecoxib (60.64%) when administered at 100 mg/kg (76.86%). This result was aimed at all candidates who had long-lasting activity greater than 50%, as represented by most anti-inflammatory protocols. The results showed that all groups did not exert any significant activity on edema inhibition as compared to reference drugs diclofenac and celecoxib (58.18 and 41.97%, respectively) before 1 hr.

The 1st onset of action is seen after 2 hours of carrageenan injection and 2.5 hours after candidates' administration, where either ESA-EO (50 and 100 mg/kg) or ESA-EO-NE (50 and 100 mg/kg) exerted moderate-to-strong edema inhibition percentages (46.98 and 78.51%; 72.75 and 58.64%, respectively). These activities were higher than or equal to the reference drugs diclofenac and celecoxib (89.02 and 78.36%, respectively). This edema inhibitory effect continued to be the best action at 3 hrs intervals in the case of all candidates, including the reference drug (diclofenac), while the other reference drug, celecoxib, was declined (Table 1S; Figure 4).

3.4.3. Effect on GSH, MDA, and Nitric Oxide (NO). A substantial increase in the level of MDA and a decrease in GSH content were observed as a result of carrageenan injections, with the respective means of 1.95 and 0.45 μmol/mg tissue, as compared to means of the normal control (0.33 and 1.18 μmol/mg tissue, respectively). It is noticed that MDA was reduced in the groups treated with ESA-EO (50 and 100 mg/kg) and ESA-EO-NE (50 and 100 mg/kg) through 0.85, 0.48, 0.42, and 0.37 nmol/mg tissue, respectively, as compared to the carrageenan group's mean (Table 2). Additionally, the treatment with ESA-EO and ESA-EO-NE (50 and 100 mg/kg) has retained the antioxidant state by increasing GSH content utilizing 0.51, 0.70, 0.79, and 0.88 μmol/mg tissue, respectively, as compared to the mean of the carrageenan group (Table 2). It is observed that both doses of ESA-EO-NE have significantly reduced MDA levels, reaching the normal control level at a high dose (100 mg/kg), and have similar or superior effects to both reference drugs. It is also noticed that 100 mg/kg of ESA-EO-NE has significantly restored the normal contents of GSH, with the same capacity as diclofenac and superior to celecoxib.

When compared to the normal control group, the positive control group that received 0.1 ml of carrageenan (1% w/v) injection had a substantial (p 0.05) increase in NO of 314.76%. Oral treatment with diclofenac or celecoxib (groups III and IV) significantly ($p < 0.05$) decreased the induced NO by 239.89% and 207.73%, respectively, when compared to the positive control group. Oral treatment of groups V and VI with ESA-EO (50–100 mg/kg) significantly ($p < 0.05$) decreased the induced NO by 124.78% and 187.17%, respectively, as compared to the positive control group. It is also noticed that NO was markedly ameliorated in groups treated with high doses of ESA-EO and ESA-EO-NE and reached levels near those of the normal control group. The two doses of ESA-EO-NE at 50–100 mg/kg were the highly significant candidates for decreasing the elevated NO by 238.84% and 298.07%, respectively, as compared to the positive control group (Table 2).

3.4.4. Effect on Inflammatory Cytokines. A substantial increase in the levels of TNF-α was observed as a result of carrageenan injections with 0.1 ml (1% w/v) (125.8 ± 1.20)

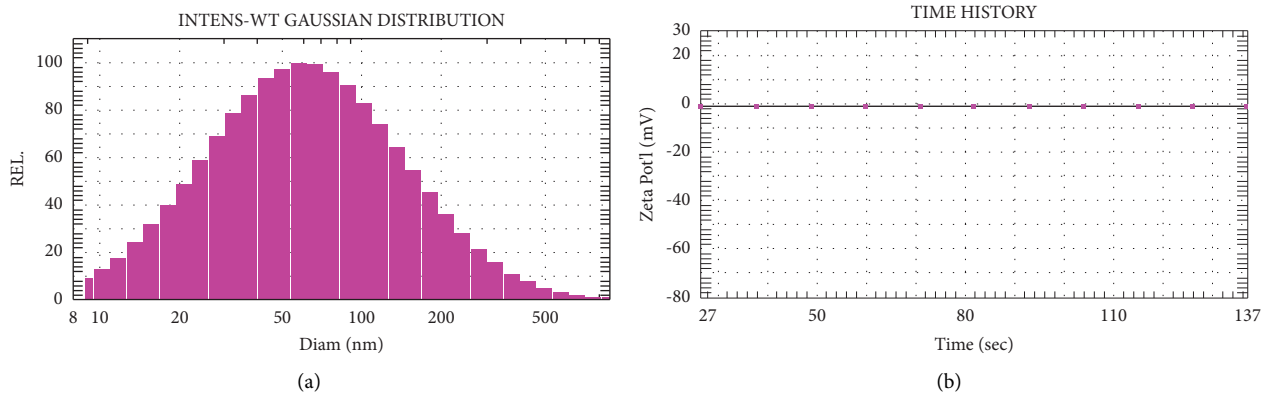

Figure 3: *Eugenia supra-axillaris*shoots EO nanoemulsion (ESA-EO-NE) particle size distribution ((a), mean diameter = 88 nm) and zeta analysis (b).

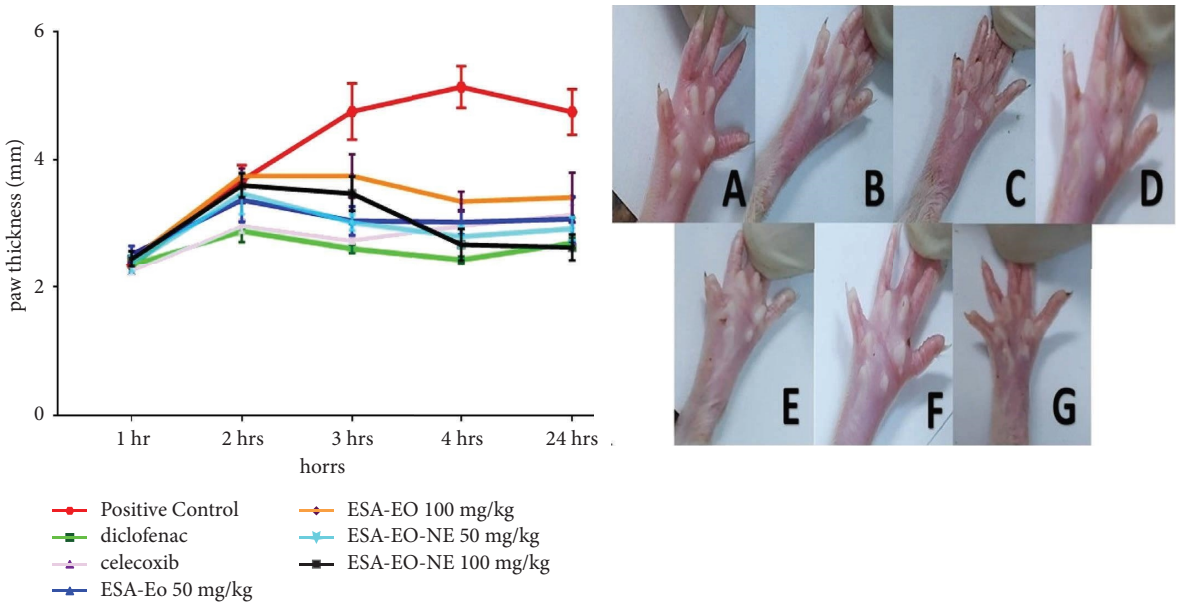

Figure 4: Effect of oral administration of ESA-EO (50–100 mg/kg) and ESA-EO-NE (50–100 mg/kg) on the mean of paw thickness (carrageenan-induced paw edema in rats) after 1, 2, 3, 4, and 24 h of the tested drug administration. Data are expressed as mean ± SE. Positive control (a), diclofenac (30 mg/kg) (b), celecoxib (50 mg/kg) (c), ESA-EO (50 mg/kg) (d), ESA-EO (100 mg/kg) (e), ESA-EO-NE (50 mg/kg) (f), and ESA-EO-NE (100 mg/kg) (g).

pg/ml as compared to the normal control group (41.77 ± 1.39) pg/ml (Figure 5(a)). It is noticed that oral treatment with standard drugs, diclofenac and celecoxib (groups III and IV), significantly ($p < 0.05$) decreased the induced TNF-α (48.71 ± 0.48 and 64.13 ± 1.68 pg/ml, respectively) when compared to the positive control group. Oral treatment of groups (4–7) with ESA-EO and ESA-EO-NE (50–100 mg/kg), respectively, significantly decreased the elevated TNF-α (62.6 ± 1.47, 51.21 ± 1.44, 48.29 ± 0.96, and 43.29 ± 0.48 pg/ml, respectively) as compared to the positive control group (Figure 5(a)).

3.4.5. Quantification of Prostaglandin E2 (PGE2) RNA Expression. In the tissue homogenate, prostaglandin E2 (PGE2) RNA expression was evaluated. When compared to the normal control group (1.03 ± 0.06 copies x 10^4/mg total protein), the results demonstrated a significantly higher level of PGE2 RNA expression ($p < 0.05$) in the positive control group (3.16 ± 0.07 copies x 10^4/mg total protein) (Figure 5(b)). When compared to the positive control group, oral administration of diclofenac (150 mg/kg) resulted in a substantial decrease ($p < 0.05$) in PGE2 RNA expression (1.59 ± 0.03 copies x 10^4/mg total protein). In comparison to the positive control group, oral administration of ESA-EO and ESA-EO-NE (50 and 100 mg/kg) showed a substantial reduction ($p < 0.05$) in PGE2 RNA expression (1.89 ± 0.08, 1.69 ± 0.01, 1.33 ± 0.004, and 1.29 ± 0.008 copies x 10^4/mg total protein, respectively) (Figure 5(b)).

3.5. Results of Analgesic Activity. It is revealed that high doses (100 mg/kg) of ESA-EO and ESA-EO-NE showed about the same analgesic efficacy after 90 minutes of administration,

Table 2: Effect of oral administration of ESA-EO and ESA-EO-NE on GSH, MDA, and NO.

Group	GSH (μmol/mg tissue)	MDA (nmol/mg tissue)	NO (Mmol/L)
Negative control	1.183 ± 0.05^{bcd}	0.329 ± 0.03^{bcd}	14.37 ± 0.46^{bcd}
Carrageenan	0.449 ± 0.02^{ac}	1.95 ± 0.06^{acd}	59.60 ± 01.34^{acd}
Diclofenac (30 mg/kg)	0.852 ± 0.04^{ab}	0.538 ± 0.02^{abd}	25.13 ± 01.08^{ab}
Celecoxib (50 mg/kg)	0.538 ± 0.04^{a}	0.840 ± 0.02^{abc}	29.75 ± 0.17^{ab}
ESA-EO (50 mg/kg)	0.513 ± 0.02^{ac}	0.851 ± 0.04^{abc}	41.67 ± 0.87^{abcd}
ESA-EO (100 mg/kg)	0.703 ± 0.05^{ac}	0.478 ± 0.02^{abd}	32.70 ± 0.46^{ab}
ESA-EO-NE (50 mg/kg)	0.785 ± 0.04^{ab}	0.417 ± 0.02^{bcd}	25.28 ± 0.58^{ab}
ESA-EO-NE (100 mg/kg)	0.88 ± 0.05^{b}	0.371 ± 0.01^{bcd}	16.77 ± 0.22^{bd}

Data are expressed as mean ± SE where $n = 6$. Statistics were carried out by one-way ANOVA and confirmed by Tukey's multiple comparison test. $p < 0.05$ was assumed to denote statistical significance. [a]Statistically significant from the control negative group. [b]Statistically significant from the carrageenan group. [c]Statistically significant from the diclofenac group. [d]Statistically significant from the celecoxib group.

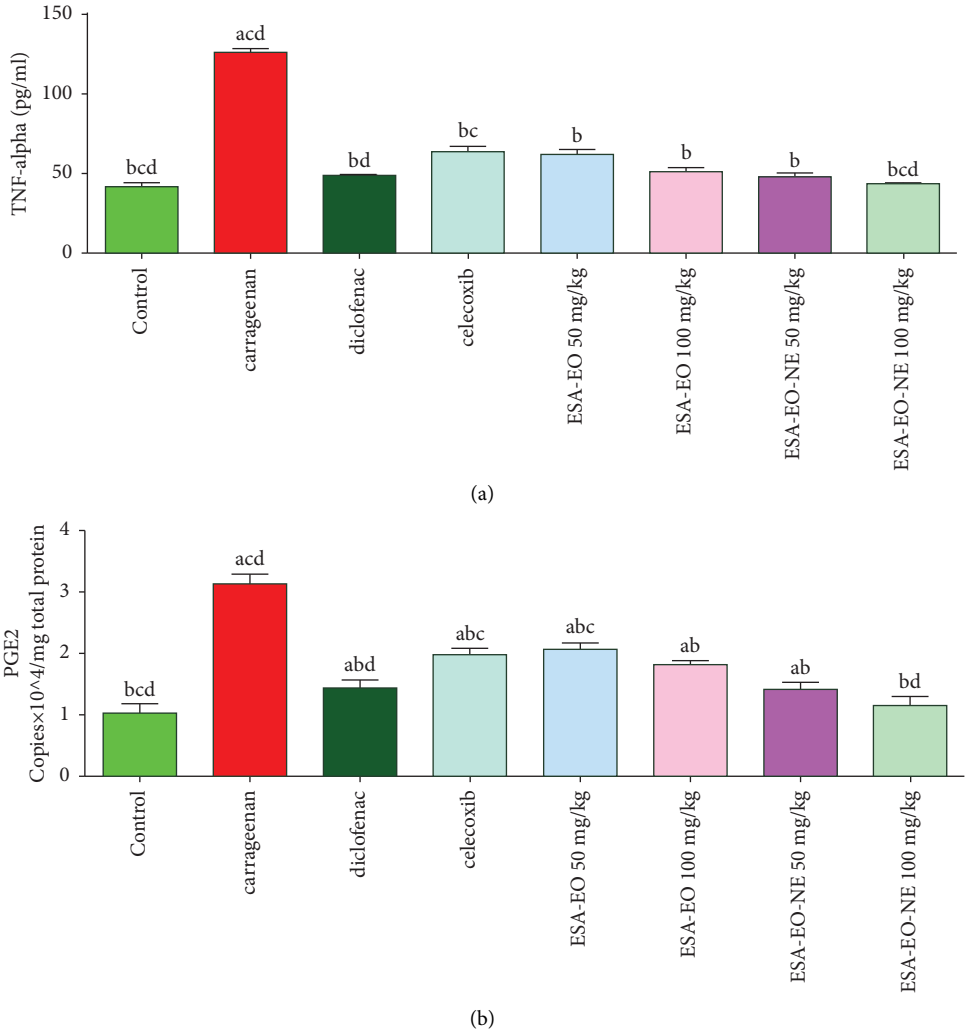

Figure 5: Effect of oral administration of ESA-EO and ESA-EO-NE on TNF-α (a), and PGE2 (b), as compared to the standard drug, diclofenac (30 mg/kg) and celecoxib (50 mg/kg). Data are expressed as mean ± SE where $n = 6$. Statistics were carried out by one-way ANOVA and confirmed by Tukey's multiple comparison test. $p < 0.05$ was assumed to denote statistical significance. [a]Statistically significant from the control negative group. [b]Statistically significant from the carrageenan group. [c]Statistically significant from the diclofenac group. [d]Statistically significant from the celecoxib group.

with percentage of pain inhibition of 100.74 and 101.18%, respectively, compared with both reference drugs diclofenac and celecoxib (% of pain inhibition = 71.32 and 77.65 respectively) while both low doses (50 mg/kg) of ESA-EO and ESA-EO-NE showed minimal percentages of pain inhibition (48.24% and 66.18%, respectively). On the other hand, only the high dose (100 mg/kg) of ESA-EO-NE showed a fast onset of action in the 1st 30 min of drug administration with

a percentage of pain inhibition of 99.55%, while the high dose (100 mg/kg) of ESA-EO showed 76.48%, in comparison with both reference drugs diclofenac and celecoxib (% of pain inhibition = 70.79 and 65.54, respectively) (Table 3).

3.6. Results of Antipyretic Effects. After 24 hours, a brewer's yeast suspension intramuscular injection markedly increased the rectal temperature. Up until 90 min after intramuscular injection of brewer's yeast, oral administration of the ESA-EO and ESA-EO-NE in both doses (50 and 100 mg/kg) demonstrated a gradual reduction in rectal temperature, whereas oral administration of the reference drugs diclofenac (30 mg/kg) and celecoxib (50 mg/kg) continued to reduce fever until the final measurement (120 min). All of the treatments were shown to significantly (p 0.05) lower the temperature when compared to the positive control group in all of the groups (Figure 6, Table 2S).

The main reproducible inflammatory model was previously characterized as the paw edema brought on by carrageenan. The results of the current investigation showed that the ESA-EO and ESA-EO-NE can effectively reduce the inflammation that carrageenan-induced paw edema causes. The boosting and/or inhibiting of inflammatory cytokine responses were directly linked with the progression of the inflammatory paw edema [37–40]. The results of this study showed that TNF-α, NO, and PGE2 levels were dramatically reduced by ESA-EO and ESA-EO-NE. In addition to activating macrophages and inducing the release of other cytokines such as IL-1 and IL-6, TNF-α is one of the primary inflammatory mediators that control T-cell immunological responses. [41]. These actions backed up the current findings that show the efficacy of ESA-EO and ESA-EO-NE in treating associated inflammation-related disorders such as pyrexia and pains.

Generally, a hot plate is the most suitable method for the pain response assessment in animals to evaluate centrally acting analgesic; in contrast, the peripheral acting analgesic could be only evaluated by an acetic acid-induced writhing test. Different mechanisms were incorporated into the pain transmission and pain reduction process [24, 42]. PGE2 plays a crucial role in the regulation of pain and inflammation. It is synthesized by the cyclooxygenase (COX) pathway from arachidonic acid, which is released from cell membranes in response to tissue injury or inflammation. PGE2 acts on specific receptors located in sensory neurons, glial cells, and immune cells to modulate pain transmission and perception [43]. Also, TNF-α and NO are two important molecules that play a crucial role in central analgesia [44]. The tissue content of TNF-α and NO can affect central analgesia in several ways. Firstly, TNF-α can sensitize nociceptive neurons in the spinal cord by increasing their excitability and reducing their threshold for activation. This can lead to an amplification of pain signals and contribute to the development of chronic pain conditions [45]. The hot plate assaying results revealed that the ESA-EO and ESA-EO-NE can increase latency time as a positive response in reducing the nociceptive threshold.

The biological activities of the natural plant extracted were directly attributed to their bioactive metabolites [17]. Here, the principal identified components, including mono- and sesquiterpenes, could be attributed to ESA-EO's high potentiality against inflammation, pyretic, and pains. Numerous studies have shown that monoterpenoids are powerful anti-inflammatory compounds [42]. Herein, d-limonene and α-pinene were found as the main compounds with relative concentrations over 57% of the total mass of the EO. These two compounds were described in several published data as active anti-inflammation mediators [43]. Several EOs derived from several plants were described to have significant potentialities against inflammations, pyretic in addition to analgesic due to the strong contribution of d-limonene and α-pinene as main active components such as *Araucaria heterophylla* [17], *Araucaria bidwillii* [18], *Helichrysum odoratissimum*, *Heteropyxis natalensis*, and *Lippia javanica* [42]. These two compounds were described to inhibit inflammation and paw edema via the suppression of the production of leukotriene, the eicosanoid inflammation mediator, that supported their abilities for inhibiting various inflammatory complications [44, 45]. Both compounds were also reported to have the capability for inhibition of the 5-lipoxygenase enzyme, the activity of neutrophils, and thus decreasing the probability of inflammation reactions [46, 47]. Present findings went in the same pathway via these inflammation inhibitors. Additionally, the ESA-EO and ESA-EO-NE might play as immunomodulators via the reduction of inflammation mediator's secretion by the chemical components that stop the cytokines production.

Furthermore, the observed outcomes along with the documented data corroborated the powerful anti-inflammatory effects of sesquiterpenoids [42]. As the main component of the EOs of various plants such as *Ageratum fastigiatum* [46, 48], *Araucaria heterophylla* [49], and *Araucaria bidwillii* [18], germacrene-D, one of the important sesquiterpenes in the current investigation, was reported to have considerable anti-inflammation effects. In the study of Fernandes et al. [50], the isolated α-humulene and *trans*-caryophyllene from the EO of *Cordia verbenacea* along with α-cadinol were described to display significant *in vivo* anti-inflammatory effects. These compounds were reported to reduce the carrageenan-paw edema and platelet activation factor via the inhibition of TNF-α, PGE-2, nitric oxide synthase (NO), and other interleukins [50]. Current results were found to move in complete harmony with the data of Fernandes et al. [50], where these two compounds represented major constituents. In general conclusion of this point, the ESA-EO chemical constituents, majors and minors, might act as inflammation inhibitory agents via singular action and/or synergetic contributions.

The nanoemulsion became one of the significant strategies for increasing the stability of EOs along with increasing their bioactivities via decreasing volatility and increasing their cellular absorption [16]. This theory was confirmed by several scientific works, such as EO-NEs of *Araucaria bidwillii*, *Araucaria heterophylla*, *Rosmarinus officinalis*, *Cinnamomum zeylanicum*, and *Syzygium aromaticum* [17, 18, 41, 51, 52].

Table 3: Central analgesic efficiency of the ESA-EO and ESA-EO-NE.

Group	30 min.	60 min.	90 min.	120 min.
Negative control	6.68 ± 1.29 (--)	6.6 ± 0.089 (--)[dc]	6.8 ± 0.84 (--)[dc]	8.28 ± 0.67
Diclofenac (30 mg/kg)	11.4 ± 1.64 (70.79)	12.15 ± 0.25 (84.09)[a]	11.65 ± 0.79 (71.32)[a]	9.23 ± 1.58 (11.48)
Celecoxib (50 mg/kg)	11.05 ± 0.82 (65.54)	11.63 ± 0.83 (76.21)[a]	12.08 ± 1.02 (77.65)[a]	9.95 ± 0.88 (20.24)
ESA-EO (50 mg/kg)	10.4 ± 1.11 (55.81)	11.9 ± 1.38 (80.30)[a]	10.08 ± 1.34 (48.24)	8.53 ± 1.29 (3.02)
ESA-EO (100 mg/kg)	11.78 ± 1.27 (76.48)	12.3 ± 0.60 (86.36)[a]	13.65 ± 0.53 (100.74)[a]	10.23 ± 0.60 (23.63)
ESA-EO-NE (50 mg/kg)	10.36 ± 1.29 (55.21)	11.83 ± 0.45 (79.24)[a]	11.3 ± 0.91 (66.18)[a]	10.63 ± 1.68 (28.46)
ESA-EO-NE (100 mg/kg)	13.32 ± 0.15 (99.55)[a]	12.88 ± 0.13 (95.15)[a]	13.68 ± 0.23 (101.18)[a]	11.88 ± 1.84 (43.56)

Data are expressed as mean ± SE where $n = 6$. Statistics were carried out by one-way ANOVA and confirmed by Tukey's multiple comparison tests. $p < 0.05$ was assumed to denote statistical significance. [a]Statistically significant from the control negative group. [c]Statistically significant from the diclofenac group. [d]Statistically significant from the celecoxib group.

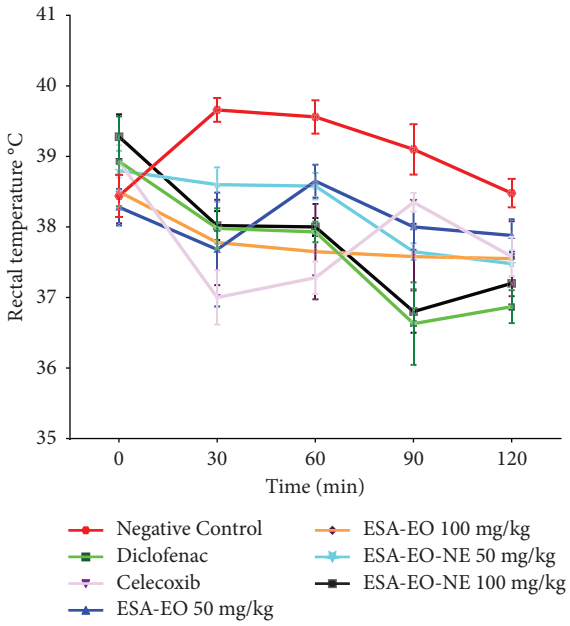

Figure 6: Effect of oral administration of ESA-EO (50–100 mg/kg) and ESA-EO-NE (50–100 mg/kg) on pyrogenesis induced by intramuscular injection of rats with brewer's yeast as compared to standard drug; diclofenac (30 mg/kg) and celecoxib (50 mg/kg). Data are expressed as mean ± SE. Statistics were carried out by one-way ANOVA and confirmed by Tukey's test. $p < 0.05$ was assumed to denote statistical significance.

Herein, the results revealed that the ESA-EO-NE is more active than the ESA-EO in both concentrations as analgesic, antipyretic, and anti-inflammatory agents in a dose-dependent manner.

4. Conclusions

A nanoemulsion of *E. supra-axillaris's* EO was prepared after it had been isolated and chemically analysed using GC-MS which revealed the abundance of the mono- and sesquiterpenes. Rats were used to assess the potential antipyretic and central analgesic effects of essential oil and its nanoformulation as well as the *in vivo* inflammation suppression effectiveness against carrageenan-induced models. Compared to the control and reference medicines, the administration of essential oil and its nanoformulation demonstrated considerable long-lasting anti-inflammatory activity in the rat paw edema model. Significantly lessened levels of proinflammatory cytokines, TNF-α, NO, and PGE2, in paw tissues, as well as the improved antioxidant status of the paw, all contributed to the significant reduction in inflammation. A substantial analgesic and antipyretic efficacy was additionally detected in rats administered essential oil and its nanoformulation orally. These findings suggested the potential therapeutic applications for *E. supra-axillaris's* EO and its nanoemulsion for treating a variety of painful and inflammatory conditions as well as hyperthermia-related illnesses.

Data Availability

All data are already available in the manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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Supplementary Materials

Table 1S: anti-inflammatory effect and percentage of edema inhibition of the ESA-EO and ESA-EO-NE on carrageenan-induced paw edema in rats after 1, 2, 3, 4, and 24 h of the tested drug administration. Table 2S: antipyretic activity of ESA-EO and ESA-EO-NE in yeast-induced pyrexia in rats. (*Supplementary Materials*)

References

[1] M. I. Yatoo, A. Gopalakrishnan, A. Saxena et al., "Anti-inflammatory drugs and herbs with special emphasis on herbal medicines for countering inflammatory diseases and disorders-a review," *Recent Patents on Inflammation and Allergy Drug Discovery*, vol. 12, no. 1, pp. 39–58, 2018.

[2] J. M. Bennett, G. Reeves, G. E. Billman, and J. P. Sturmberg, "Inflammation–nature's way to efficiently respond to all types of challenges: implications for understanding and managing "the Epidemic"of chronic diseases," *Frontiers of Medicine*, vol. 5, p. 316, 2018.

[3] M. Gupta, N. Singh, M. Gulati, R. Gupta, K. Sudhakar, and B. Kapoor, "Herbal bioactives in treatment of inflammation:

an overview," *South African Journal of Botany*, vol. 143, pp. 205–225, 2021.

[4] S. Harirforoosh, W. Asghar, and F. Jamali, "Adverse effects of nonsteroidal antiinflammatory drugs: an update of gastrointestinal, cardiovascular and renal complications," *Journal of Pharmacy and Pharmaceutical Sciences*, vol. 16, no. 5, pp. 821–847, 2014.

[5] A. C. Wiseman, "Immunosuppressive medications," *Clinical Journal of the American Society of Nephrology*, vol. 11, no. 2, pp. 332–343, 2016.

[6] M. M. Al Qaraghuli, A. R. Alzahrani, K. Niwasabutra, M. A. Obeid, and V. A. Ferro, "Where traditional drug discovery meets modern technology in the quest for new drugs," *Annals of Pharmacology and Pharmaceutics*, vol. 2, pp. 1–5, 2017.

[7] F. Jamshidi-Kia, Z. Lorigooini, and H. Amini-Khoei, "Medicinal plants: past history and future perspective," *Journal of Herbmed Pharmacology*, vol. 7, no. 1, pp. 1–7, 2018.

[8] E. A. Aboutabl, K. M. Meselhy, E. M. Elkhreisy, M. I. Nassar, and R. Fawzi, "Composition and bioactivity of essential oils from leaves and fruits of *Myrtus communis* and *Eugenia supraxillaris* (Myrtaceae) grown in Egypt," *Journal of Essential Oil Bearing Plants*, vol. 14, no. 2, pp. 192–200, 2011.

[9] M. S. Baliga, H. P. Bhat, B. R. V. Baliga, R. Wilson, and P. L. Palatty, "Phytochemistry, traditional uses and pharmacology of *Eugenia jambolana* Lam.(black plum): a review," *Food Research International*, vol. 44, no. 7, pp. 1776–1789, 2011.

[10] K. K. Bhargava, R. Dayal, and T. R. Seshadri, "Chemical components of *Eugenia jambolana* stem bark," *Current Science*, vol. 39, 1974.

[11] C. D. Bannon, R. A. Eade, and J. J. H. Simes, "Extractives of Australian timbers. XVI. The constituents of the wood of *Eugenia crebrinervis* (syn. *Syzigium crebrinerve*) and *Eugenia gustavioides* (syn. *Cleistocalyx gustavioides*)," *Australian Journal of Chemistry*, vol. 29, no. 5, pp. 1135–1141, 1976.

[12] M. El Raey, P. Proksch, M. Nawwar, and H. Barakat, "New sulphated rhamnoside of ellagic acid monomethylether from *Eugenia supra-axillaris* of potent antioxidant activity," *Planta Medica*, vol. 80, no. 16, p. LP30, 2014.

[13] H. H. Barakat, M. El-Raey, S. A. Nada, I. Zeid, and M. Nawwar, "Constitutive phenolics and hepatoprotective activity of *Eugenia supra-axillaris* leaves," *European Journal of Advanced Chemistry*, vol. 54, pp. 313–323, 2011.

[14] N. M. Hegazi, M. Sobeh, S. Rezq et al., "Characterization of phenolic compounds from *Eugenia supra-axillaris* leaf extract using HPLC-PDA-MS/MS and its antioxidant, anti-inflammatory, antipyretic and pain killing activities *in vivo*," *Scientific Reports*, vol. 9, no. 1, Article ID 11122, 2019.

[15] M. É. A. Stefanello, A. C. R. F. Pascoal, and M. J. Salvador, "Essential oils from neotropical Myrtaceae: chemical diversity and biological properties," *Chemistry and Biodiversity*, vol. 8, no. 1, pp. 73–94, 2011.

[16] A. R. Bilia, C. Guccione, B. Isacchi, C. Righeschi, F. Firenzuoli, and M. C. Bergonzi, "Essential oils loaded in nanosystems: a developing strategy for a successful therapeutic approach," *Evidence-based Complementary and Alternative Medicine*, vol. 2014, Article ID 651593, 14 pages, 2014.

[17] A. I. Elshamy, N. M. Ammar, H. A. Hassan et al., "Essential oil and its nanoemulsion of *Araucaria heterophylla* resin: chemical characterization, anti-inflammatory, and antipyretic activities," *Industrial Crops and Products*, vol. 148, Article ID 112272, 2020.

[18] M. F. Abdelhameed, G. F. Asaad, T. I. M. Ragab et al., "Oral and topical anti-inflammatory and antipyretic potentialities of *Araucaria bidiwillii* shoot essential oil and its nanoemulsion in relation to chemical composition," *Molecules*, vol. 26, no. 19, p. 5833, 2021.

[19] R. V. BandeiraReidel, S. Nardoni, F. Mancianti et al., "Chemical composition and antifungal activity of essential oils from four Asteraceae plants grown in Egypt," *Zeitschrift für Naturforschung C*, vol. 73, no. 7-8, pp. 313–318, 2018.

[20] F. A. Araújo, R. G. Kelmann, B. V. Araújo, R. B. Finatto, H. F. Teixeira, and L. S. Koester, "Development and characterization of parenteral nanoemulsions containing thalidomide," *European Journal of Pharmaceutical Sciences*, vol. 42, no. 3, pp. 238–245, 2011.

[21] A.-T. H. Mossa, S. I. Afia, S. M. M. Mohafrash, and B. A. Abou-Awad, "Formulation and characterization of garlic (*Allium sativum* L.) essential oil nanoemulsion and its acaricidal activity on eriophyid olive mites (Acari: eriophyidae)," *Environmental Science and Pollution Research*, vol. 25, no. 11, pp. 10526–10537, 2018.

[22] S. S. El-Hawary, W. E. Hammam, M. El-Mahdy El-Tantawi et al., "Apple leaves and their major secondary metabolite phlorizin exhibit distinct neuroprotective activities: evidence from in vivo and in silico studies," *Arabian Journal of Chemistry*, vol. 14, no. 6, Article ID 103188, 2021.

[23] S. S. Abd El-Karim, H. S. Mohamed, M. F. Abdelhameed, A. El-Galil E Amr, A. A. Almehizia, and E. S. Nossier, "Design, synthesis and molecular docking of new pyrazole-thiazolidinones as potent anti-inflammatory and analgesic agents with TNF-α inhibitory activity," *Bioorganic Chemistry*, vol. 111, Article ID 104827, 2021.

[24] A. M. Ghanim, A. S. Girgis, B. M. Kariuki et al., "Design and synthesis of ibuprofen-quinoline conjugates as potential anti-inflammatory and analgesic drug candidates," *Bioorganic Chemistry*, vol. 119, Article ID 105557, 2022.

[25] H. A. Hassan, I. M. Ayoub, T. I. M. Ragab et al., "Metabolomics approach of *Symphyotrichum squamatum* ethanol extract and its nano-Ag formulation protective effect on gastric ulcer via bio-chemical and pathological analyses," *Biomarkers*, vol. 28, no. 2, pp. 190–205, 2023.

[26] A. M. Srour, H. H. Fahmy, M. A. Khater, E. S. Zarie, S. S. Mohamed, and M. F. Abdelhameed, "Synthesis, anti-inflammatory properties, molecular modelling and potential COX-2, TNF-α, PGE2 and IL1β inhibitors of pyrazole-based scaffolds," *Journal of Molecular Structure*, vol. 1266, Article ID 133499, 2022.

[27] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Analytical Biochemistry*, vol. 72, no. 1-2, pp. 248–254, 1976.

[28] K. M. Miranda, M. G. Espey, and D. A. Wink, "A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite," *Nitric Oxide*, vol. 5, no. 1, pp. 62–71, 2001.

[29] J. K. R. Da Silva, E. H. A. Andrade, L. H. Barreto et al., "Chemical composition of four essential oils of *Eugenia* from the Brazilian Amazon and their cytotoxic and antioxidant activity," *Medicine (Baltimore)*, vol. 4, no. 3, p. 51, 2017.

[30] G. Oboh, I. A. Akinbola, A. O. Ademosun et al., "Essential oil from clove bud (*Eugenia aromatica* Kuntze) inhibit key enzymes relevant to the management of type-2 diabetes and some pro-oxidant induced lipid peroxidation in rats pancreas in vitro," *Journal of Oleo Science*, vol. 64, no. 7, pp. 775–782, 2015.

[31] H. Özbek and A. Öztürk, "The Anti-Inflammatory Activity of *Eugenia Caryophllata* essential oil: an animal model of anti-inflammatory activity," *Electronic Journal of General Medicine*, vol. 2, no. 4, pp. 159–163, 2005.

[32] D. L. Santos, H. D. Ferreira, L. L. Borges et al., "Chemical composition of essential oils of leaves, flowers and fruits of *Hortia oreadica*," *Revista Brasileira de Farmacognosia*, vol. 26, no. 1, pp. 23–28, 2016.

[33] L. T. Huong, T. D. Thang, and I. A. Ogunwande, "Chemical constituents of essential oils from the leaves, stems, roots and fruits of *Alpinia polyantha*," *Natural Product Communications*, vol. 10, no. 2, Article ID 1934578X1501000, 2015.

[34] A. E.-N. G. El Gendy, A. F. Essa, A. A. El-Rashedy et al., "Antiviral potentialities of chemical characterized essential oils of *Acacia nilotica* bark and fruits against hepatitis a and herpes simplex viruses: in vitro, in silico, and molecular dynamics studies," *Plants*, vol. 11, no. 21, p. 2889, 2022.

[35] J. Bałdyga, W. Orciuch, Ł. Makowski, M. Malski-Brodzicki, and K. Malik, "Break up of nano-particle clusters in high-shear devices," *Chemical Engineering and Processing: Process Intensification*, vol. 46, no. 9, pp. 851–861, Article ID 12007, 2007.

[36] C. Fernandes, F. de Almeida, A. Silveira et al., "Development of an insecticidal nanoemulsion with *Manilkara subsericea* (Sapotaceae) extract," *Journal of Nanobiotechnology*, vol. 12, no. 1, p. 22, 2014.

[37] M. Feldmann, F. M. Brennan, and R. N. Maini, "Role of cytokines in rheumatoid arthritis," *Annual Review of Immunology*, vol. 14, no. 1, pp. 397–440, 1996.

[38] D. N. Mannel and B. Echtenacher, "TNF in the inflammatory response," *CD14 Inflamm Response*, vol. 74, pp. 141–161, 2000.

[39] Z. Halici, G. O. Dengiz, F. Odabasoglu, H. Suleyman, E. Cadirci, and M. Halici, "Amiodarone has anti-inflammatory and anti-oxidative properties: an experimental study in rats with carrageenan-induced paw edema," *European Journal of Pharmacology*, vol. 566, no. 1-3, pp. 215–221, 2007.

[40] V. P. Nacife, M. D. N. C. Soeiro, R. N. Gomes, H. D'Avila, H. C. C.-F. Neto, and M. D. N. L. Meirelles, "Morphological and biochemical characterization of macrophages activated by carrageenan and lipopolysaccharide in vivo," *Cell Structure and Function*, vol. 29, no. 2, pp. 27–34, 2004.

[41] C.-H. Liao, S.-J. Guo, and J.-Y. Lin, "Characterisation of the chemical composition and *in vitro* anti-inflammation assessment of a novel lotus (*Nelumbo nucifera* Gaertn) plumule polysaccharide," *Food Chemistry*, vol. 125, no. 3, pp. 930–935, 2011.

[42] R. de Cássia da Silveira e Sá, L. N. Andrade, and D. P. de Sousa, "A review on anti-inflammatory activity of monoterpenes," *Molecules*, vol. 18, no. 1, pp. 1227–1254, 2013.

[43] G. F. Asaad, M. F. Abdelhameed, M. A. El Raey et al., "*Citrus clementine* peels essential oil exhibited anti-SARS-CoV-2 and its modulatory effect against cytokine storm: evidence from in vitro and in silico studies," *Egyptian Journal of Chemistry*, vol. 65, pp. 419–427, 2022.

[44] G. S. Ahn, H. U. Jang, G. Y. Jung et al., "Essential oil component having inhibition activity of leukotriene production," *Korea Pat*, vol. 2102837321, no. 12, 2001.

[45] J. Yang, W.-S. Choi, K.-J. Kim, C.-D. Eom, and M.-J. Park, "Investigation of active anti-inflammatory constituents of essential oil from *Pinus koraiensis* (Sieb. et Zucc.) wood in LPS-stimulated RBL-2H3 cells," *Biomolecules*, vol. 11, no. 6, p. 817, 2021.

[46] M. Basholli-Salihu, R. Schuster, A. Hajdari et al., "Phytochemical composition, anti-inflammatory activity and cytotoxic effects of essential oils from three *Pinus* spp," *Pharmaceutical Biology*, vol. 55, no. 1, pp. 1553–1560, 2017.

[47] S. Baylac and P. Racine, "Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts," *International Journal of Aromatherapy*, vol. 13, no. 2-3, pp. 138–142, 2003.

[48] G. Del-Vechio-Vieira, O. V. D. Sousa, M. A. Miranda, L. Senna-Valle, and M. A. C. Kaplan, "Analgesic and anti-inflammatory properties of essential oil from *Ageratum fastigiatum*," *Brazilian Archives of Biology and Technology*, vol. 52, no. 5, pp. 1115–1121, 2009.

[49] A. M. Abd-ElGawad, I. Saleh, M. H. A. El-Razek et al., "Chemical profiling of significant antioxidant and phytotoxic microwave-extracted essential oil from *Araucaria heterophylla* resin," *Separations*, vol. 10, no. 2, p. 141, 2023.

[50] E. S. Fernandes, G. F. Passos, R. Medeiros et al., "Anti-inflammatory effects of compounds alpha-humulene and (−)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*," *European Journal of Pharmacology*, vol. 569, no. 3, pp. 228–236, 2007.

[51] R. S. Borges, H. Keita, B. L. S. Ortiz et al., "Anti-inflammatory activity of nanoemulsions of essential oil from *Rosmarinus officinalis* L.: *in vitro* and in zebrafish studies," *Inflammopharmacology*, vol. 26, no. 4, pp. 1057–1080, 2018.

[52] A. N. Daniel, S. M. Sartoretto, G. Schmidt, S. M. Caparroz-Assef, C. A. Bersani-Amado, and R. K. N. Cuman, "Anti-inflammatory and antinociceptive activities A of eugenol essential oil in experimental animal models," *Rev. Bras. Farmacogn.*, vol. 19, no. 1, pp. 212–217, 2009.