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# Chemical Profile, Anti-inflammatory, and Antimicrobial Activities of The Essential Oil and Lipoidal Matter Obtained from Two *Eugenia* species Cultivated in Egypt

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**Abstract**

*Eugenia* is ranked the second largest genus in family Myrtaceae and its species are distinguished by numerous health and economical significance. The current study aimed to comparatively investigate the anti-inflammatory and antimicrobial activities of the essential oils (EOs) and lipoidal matters (LMs) derived from *Eugenia uniflora* L. (EU) and *Eugenia supraaxillaris* Spring ex Mart. (ES) leaves cultivated in Egypt for the first time. The oils were obtained by the hydrodistillation (HD) and supercritical fluid solid-phase micro-extraction (SF-SPME) methods, while the lipoidal matters were extracted using petroleum ether. The GC/MS analysis of HD EO showed that curzerene (39.85%) was the abundant constituent in EU, while germacrene D (16.86%) in ES. The SF-SPME EOs showed that (*E*)-2-hexenal is a chief component in both species representing 93.87% (EU) and 46.68 % (ES). On the other side, EU LM was pioneered with hydrocarbons (95.26%) and to a lesser extent with sterols (4.08%), while both classes were evenly tracked in the LM of ES. Lastly, lauric (13.74-17.76%) and oleic (4.26-3.57%) were the most identified fatty acids in both species. The results of the bioactivity screening showed that LM of ES demonstrated the best anti-inflammatory activity through inhibiting cyclooxygenase-2 enzyme with $IC_{50}$ 0.13 µL/mL followed by its LM with $IC_{50}$ 0.135 mg/mL, while EU samples showed moderate activity. Likewise, EOs of both species displayed antimicrobial potential on all tested organism with MIC>40 µL/mL, while their LMs displayed moderate activity (MIC≥200 mg/mL). The observed activities may be possibly, at least in part, to the synergism between the individual metabolites of the EOs and LMs. In all, our study endorses the promising potential of the EOs and LMs from both *Eugenia* species in the management of microbial infection in addition to related inflammatory disorders.

*Keywords*: Anti-inflammatory; Antimicrobial; Eugenia; Essential oil; Lipoidal matter

## 1. Introduction

Since ancient times, natural compounds have been essential to human health, particularly as anti-inflammatory and antibacterial agents [1]. Inflammation is an evolutionarily preserved process of defense that is consisted of complicated subsequent modifications inside the human's tissue to remove the primary source of injury, which may be infectious, physical, or chemical origin [2]. This complicated biological response causes the recovery of homeostasis. On the other hand, in cases of extended release of inflammatory mediators and the stimulation of destructive signal-transduction pathways, the inflammatory procedure continues, and a mild or chronic pro-inflammatory condition may appear [2]. Among the common inflammatory pathways, are those which starts with the release of cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), and 5-lipoxygenase (5-LOX) enzymes. They metabolize arachidonic acid into inflammatory mediators as prostaglandins, thromboxane, and leukotrienes [3]. Several typical non-steroidal anti-inflammatory drugs, such as aspirin, apply their anti-inflammatory effect through rough inhibition of both COX-1 and COX-2, an action that is accompanied by gastric bleeding. Though several molecules have been synthesized with the objective to overcome such unfavorable profile, but their toxicity has limited them from being used in clinics. On the other side, promising pharmacophores that serves as dual inhibitors of COX-2/5-LOX enzymes, showed adjusted safety profile and efficacy. They inhibit the formation of both prostaglandins and leukotrienes and reduce tissue damage [3]. Hence, the development of new anti-inflammatory agents with a dual inhibitory activity are in dire need [4].

Another important therapeutic area witnessed the great impact of the privileged scaffolds of natural

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products, is the antimicrobial impact. The increasing prevalence of pathogenic bacteria that are resistant to presently available antibiotics is an alarming threat to public health [5]. Natural products as fats and essential oils, are typical lipophiles that can disrupt the structure of the cytoplasmic membrane and permeabilize inside various microbes with subsequent potential antimicrobial activity [6,7]. Thus, it is interesting to discover new essential oils and lipids-based-antimicrobial agents directed at antagonising these pathogens and decreasing the obtained resistance.

Eugenia is the second largest genus in family Myrtaceae, encompassing nearly 1,011 species of aromatic trees and shrubs [8,9]. Among its generic species is *Eugenia uniflora* L. (known as Pitanga cherry), a Brazilian native tree that is highly appreciated for its cherry, sweet edible fruits, and attractive, ornamental, aromatic leaves [10,11]. The leaves have been used for treating inflammatory and stomach disorders, rheumatism, fever, and hypertension [12,13], while prior studies have documented its anti-inflammatory and anti-microbial properties [12-16]. The forementioned benefits may be linked to the presence of various secondary metabolites, which include flavonoids, condensed and hydrolysable tannins, leucoanthocyanidins, steroids and/or triterpenoids, and essential oil [17]. Remarkably, *uniflora* essential oil has been integrated into the Brazilian cosmetics industry as in shampoos, hair conditioners, face and bath soaps, body oils, and perfumes [17]. In addition, reportedly that the essential oil obtained by the conventional hydro-distillation method possessed antifungal [18], antibacterial and cytotoxic, antinociceptive and hypothermic properties [17], antioxidant [19], and antileishmanial [20]. Another interesting *Eugenia* species is the *supraaxillaris* Spreng ex Mart., which is a Brazilian evergreen tree that is cultivated in tropical and subtropical countries. The species is esteemed by diverse phenolic constituents, and essential oil [21]. Yet, the leaves' essential oil was noticed for its anticancer, antiparasitic, and antioxidant activities [22-24]. Despite the previously mentioned data, the comparative chemical profile of the extracted essentials and lipoidal matters from *E. uniflora* and *E. supraaxillaris*, cultivated in Egypt, by various methods did not address yet.

In this respect, this study aimed at the comparative chemical investigation of the essential oils and lipoidal matters obtained from the leaves of *Eugenia uniflora* L. and *Eugenia supraaxillaris* Spring ex Mart. cultivated in Egypt. Moreover, evaluating their anti-inflammatory and antimicrobial activities to demonstrate the correlation between their chemical composition and the proposed bioactivity. Hence, support the possibility of their usage as natural resources in therapeutic area.

## 2. Experimental

### *2.1. Plant material*

Leaves of *Eugenia uniflora* L. and *Eugenia supraaxillaris* Spring ex Mart. were collected in October 2021 from Giza Zoo, Egypt. Both species were validated by Dr. Trease Labib, Taxonomist at Mazhar Botanical Garden, Giza, Egypt. Voucher specimens were implied as 01 Eun/2021 and 02Esu/2021 and deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

### *2.2. Extraction of the essential oils (EOs)*

#### *2.2.1. Hydrodistillation (HD)-Clevenger based extraction*

The fresh leaves of *Eugenia uniflora* L. and *Eugenia supraaxillaris* Spring ex Mart. (wt. 150 g each) were thoroughly washed, cut into small pieces, separately placed in the distillation flask of Clevenger apparatus. Each plant's leaves were submerged in 1.5 L of distilled water and bring to boil for 6 h. EOs were distilled, separated, dried over anhydrous sodium sulfate, and stored in well-sealed glass vials at 4 ºC until analyzed by GC/MS. The yield in % (v/w) was determined based on the initial plant weight [25].

#### *2.2.2. Head-space solid-phase microextraction (HS-SPME)*

About 2 g of fresh leaves of *Eugenia uniflora* L. and *Eugenia supraaxillaris* Spring ex Mart. were separately placed into 5 mL glass vial. The vial's temperature was set at 60-70 °C as an optimum temperature to saturate the vapor content in the head space of the solid surface. The solid-phase microextraction (SPME) syringe was placed in the headspace, subsequently the EOs in the vapor state, was absorbed by the silica phase in the syringe needle. After the silica fiber was sufficiently saturated with the volatile components, the fiber was directly placed into the GC/MS input section and EOs present in the fiber were adsorbed due to the temperature of the input and then entered to the GC/MS apparatus for identification [25].

### *2.3. GC/MS analysis of EOs obtained by HD method*

Mass spectra were recorded on Shimadzu GCMS-QP2020 (Tokyo, Japan) equipped with a split-spitless injector. EOs components were separated on Rtx-5MS fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, Restek, USA). Split mode injection with ratio 1: 15 was adjusted to each diluted sample (1% v/v). The apparatus was operated at the following condition: ion source temperature 220°C; injector and interface temperatures, 280°C; maximum temperature, 300°C; oven temperature program: 3 min isothermal at 50°C, then programmed from 50°-300°C at 5°C/min, followed by 10 min isothermal at 300°C; carrier gas: 1.37 mL He/min.; ion source, 70 ev MS were recorded in the range m/z 35-500 a.m.u.

*2.4. GC/MS analysis of EOs obtained by HS-SPME method*

The analysis was accomplished on Shimadzu GCMS-QP2020 NX (Tokyo, Japan) equipped with Shimadzu head space (HS)-20 sampler and split-spitless injector. EOs components were separated on Rtx-5MS fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, Restek, USA). Split mode injection with split ratio 1: 15 was adopted to each diluted sample (1% v/v). The apparatus was operated at the following condition: ion source temperature, 200°C; interface temperature, 280°C; maximum temperature, 300°C; oven temperature program: 2 min. isothermal at 45°C, then programmed from 45°-300°C at 5°C/min, followed by 5 min. isothermal at 300°C; carrier gas: 1.41 mL He/min.; ion source, 70 ev. MS were recorded in the range m/z 35-500 a.m.u.

*2.5. Identification of the EO's volatile components*

The identification of essential oil (EO) components was performed based on their recorded mass spectral data (MS) and Kovat's retention indices were calculated using a homologous array of n-alkanes (C8-C32, Sigma–Aldrich, St. Louis, MO, USA) [26]. Ultimately, values were compared to those reported on NIST (National Institute of Standards and Technology) library database in addition to previously published data [27].

*2.6. Extraction and fractionation of lipids content*

The air-dried, powdered leaves (200 g) of both species were separately extracted with pet. ether (3 x 250 mL) under reflux at 50 °C for 5 h to yield dry, brown extract. About 2 g of each extract was subjected to alkaline hydrolysis (saponification) with 50 mL of N/2 alc. potassium hydroxide under refluxed for 8 h. Thereafter, the alcohol was distilled off and the aqueous portion was shaken with pet. ether (3 x 100 mL). The aqueous layer was separated and kept aside for the derivatization step, while the pet. ether extracts were pooled, washed with distilled water until the wash was neutral to litmus paper, then the solvent was evaporated under reduced pressure to afford dark orange, semisolid extract denoted as unsaponifiable matter (USM). The USM was stored in sealed, amber vial for GC/MS analysis. On the other side, the alkaline aqueous layer remained after removal of USM was acidified with 10% sulfuric acid to liberate the free fatty acids. The latter were extracted with pet. ether (3 x 100 mL), washed several times with distilled water, then the solvent was evaporated to afford, dark brown semi-solid residue which is then subjected to derivatization *via* methylation to obtain the methylated fatty acids suitable for GLC analysis [28,29].

*2.7. Preparation of the fatty acid methyl esters (FAM)*

The fatty acids were converted to their methyl esters by refluxing with 50 mL absolute MeOH and 1.5 mL conc. $H_2SO_4$ at 50 °C for 2 h. The MeOH was distilled off and the residue was dissolved in distilled water, then extracted with pet. ether (3 x 100 mL). The extracts were pooled, washed with distilled water until the wash was neutral to litmus paper, then the solvent was evaporated under reduced pressure to afford brown, dry residue denoted as fatty acid methyl esters (FAM) [28,29]. The residue was kept in sealed, amber glass vial for GLC analysis.

*2.8. GC analysis of USM and FAM*

The GC model 7890B from Agilent Technologies was equipped with flame ionization detector at Central Laboratories Network, National Research Centre, and Cairo, Egypt. Separation was achieved using a Zebron ZB-FAME column (60 m x 0.25 mm internal diameter x 0.25 $\mu$m film thickness). Analyses were carried out using hydrogen as the carrier gas at a flow rate of 1.8 mL/min at a split-1:50 mode, injection volume of 1 µl and the following temperature program: 100 °C for 3 min; rising at 2.5 °C /min to 240 °C and held for 10 min. The injector and detector (FID) were held at 250 °C and 285 °C, respectively. Qualitative identification of the different constituents was performed by comparison of their relative retention times with those of authentic reference compound (hydrocarbons and fatty acid methyl esters) which obtained from Central Laboratories Network, National Research Centre, and Cairo, Egypt.

*2.9. Evaluation of in vitro anti-inflammatory activity*

The essential oils and lipoidal matters samples were screened for their ability to inhibit three inflammatory mediated enzymes *viz* 5-lipooxygenase, COX-1, and COX-2. The 5-Lipoxygenase (5-LOX) inhibitors screening assay kit (Cayman Chemical, Ann Arbor, MI, United States) was manipulated as stated by the manufacturer's instructions [30] with Zileuton applied as reference, standard inhibitor. On the other side, COX-1/2 were calculated by using an enzyme immunoassay (EIA) kit (Cayman Chemical, MI, USA) as stated by the manufacturer's instructions and recorded studies with celecoxib applied as reference standard, inhibitor. All data were performed in triplicates and demonstrated as $IC_{50}$±SD, which is the concentration causing 50% enzyme inhibition. Additionally, the COX-2 selectivity index (SI values) which is calculated from $IC_{50}$ (COX-1)/$IC_{50}$ (COX-2).

*2.10. Evaluation of the in vitro antimicrobial activity*

*2.10.1. Standard microbes, antibiotics, and culture media*

The stock cultures of Gram-positive bacteria as Clostridium perfringens ATCC 13124, Enterococcus faecalis ATCC 29212, and Streptococcus faecalis ATCC 8043; Gram-negative bacteria as Pseudomonas aeruginosa ATCC 9027, and Klebsiella pneumoniae ATCC 700603; fungal strains as Candida albicans ATCC 10231 and Aspergillus niger ATCC 6275) were purchased from Microlab., Institute of Research and Technology, Vellore, Tamilnadu, India. The microbiological growth media, Mueller-Hinton agar (MHA) and Mueller-Hinton broth (MHB) were gotten

from Oxoid, ThermoFisher Scientific (MA, USA). Positive control antibiotic 6.0 mm discs as Amikacin (AK; 30 µg/mL), Amoxicillin (AX; 25 µg/mL), Ampicillin/sulbactam (SAM; 20 µg/mL), Norfloxacin (NOR; 10 µg/mL), Ofloxacin (OFX; 5 µg/mL), and Nystatin (NS; 50 µg/mL) were obtained from Oxoid, ThermoFisher Scientific (MA, USA), and the biological grade sterile DMSO from ThermoFisher Scientific (MA, USA).

*2.10.2. Susceptibility test using agar diffusion assay*

The agar well-diffusion assay was carried out as per the standard method documented in the literature [31]. Briefly, 100 µl ($1\times10^5$ CFU/ml) of each reference strain suspension was seeded individually with a Muller Hinton agar (MHA) media. After solidification, about 0.6 cm diameter wells were performed with a sterile cork-borer. The wells received separately 50 µl of tested samples [32]. Consequently, the plates were incubated at 37ºC for 24 h and 25ºC for 5 days for the bacterial and fungal strains, respectively [33]. The antimicrobial susceptibility of each tested sample was calculated by determining the diameter of the inhibition zones in mm. The activity was correlated to standard antibiotics with different mechanisms of action, while diluted DMSO was used as a negative control.

*2.10.3. Broth microdilution assay*

Broth microdilution assay was accomplished as per procedure reported by Balouiri and co-workers [34]. In short, stock solutions were prepared by dissolving 100 mg of each tested sample in 1 mL DMSO. Each stock was diluted to tenths in sterile Mueller Hinton broth (MHB), thereafter 100 µl of sterile MHB was putted in each well. 150 µl of each 1/10 diluted tested sample was added in the first column of microtiter plates. Two-fold serial dilution for each 1/10 diluted extract was done by transferring 100 µl from the first to the $11^{th}$ well. 100 µl of each microbial inoculum containing $1\times10^5$ CFU/mL was transferred to all wells except blank. Then incubate all microtiter plates at 37ºC for 24 h and 25ºC for five days for inoculated bacteria and fungi strains, respectively after which the absorbances were measured at $\lambda_{max}$ 620 nm using automated microplate reader (Asys Hitech, Austria).

## 3. Results and discussion

In the current study, we investigated for the first time, the comparative phytochemical composition of the essential oils (EOs) and lipoidal matters (LMs) derived from *Eugenia uniflora* L. (EU) and *Eugenia supraaxillaris* Spring ex Mart. (ES) leaves cultivated in Egypt. Concerning the EOs, two methods were adopted *viz*, the conventional hydro-distillation (HD) and head-space solid-phase microextraction (HS-SPME). The HD, is a conventional method which uses water or steam rather than organic solvents, making it a worthy option when extraction cost is of importance [34]. Meanwhile, it involves three main physicochemical processes; hydro- diffusion, hydrolysis, and heat decomposition [35]. On the other hand, the HS-SPME technique is a relatively state-of-the-art approach for the extraction of volatiles with growing attention over the past decade. It is designed to extract volatile compounds with a wide range of boiling points with the lack of artifacts formation [36]. Though HD method was previously addressed and studied by several researchers for both species, but genetic variability, geographical, and environmental conditions directly affect the production and constitution of volatile oils [37]. Hence, this is the first comparative declaration of essential oil's analysis from both species gathered from Giza Zoo, Egypt. Our results showed that, the HD approach yielded in case of *E. uniflora* L. 0.50 %v/w colourless oil with an intense spicy-fragrant scent, while *E. supraaxillaris* yielded 0.30 %v/w colourless to pale amber EO with a faint spicy-scented odour. The extracted EOs were analyzed using GC coupled to MS with their recorded total ion chromatograms displayed as **Supplementary figures S1-S4,** and the analyzed data were delineated in **Table 1**. *E. uniflora* HD EO showed seventeen volatile components accounted for 89.66% of the total identified volatiles including oxygenated sesquiterpenes (46.00%), sesquiterpene hydrocarbons (40.55%), monoterpene hydrocarbons (0.63%), and miscellaneous components (2.51%). The most abundant oxygenated compound was curzerene (39.85%), while *δ*-guaiene (20.91%), and germacrene B (6.55%) were the major recognized hydrocarbons. Our data coincides with previous reports, especially those which highlighted that *E. uniflora* EO considered a curzerene rich source [38]. On the other hand, forty-four components were detected in *E. supraaxillaris* representing 94.06% of the total identified volatiles. The majority being categorized as non-oxygenated sesquiterpenes (56.55%), pioneered by germacrene D (16.86%), *α*-caryophyllene (11.82%), *D*-limonen (10.31%), and *α*-pinene (8.02%). Our data showed great variation in the composition and percentage of volatiles with those previously reported by Aboutabl and his research team [23] as he reported the prominence of D-limonene and *β*-pinene. The reason may be linked to the factors we mentioned earlier, in addition to the variations in the extraction conditions as the temperature. In view of hydrocarbons to oxygenated components ratio, terpene hydrocarbons are not as significant as the oxygenated compounds in the aspect of good fragrance. Oxygenated compounds are well known for their pleasant aroma [39]; hence, justify the reason behind the intense oil scent of *E. uniflora* which, at least in part, may be promoted for valuable application in fragrance industry and aromatherapy. Meanwhile, the analysis of the GC/MS data obtained from the HS-SPME oils for the first time, in comparison to the HD method, showed great variations in oil composition of both species. HS-SPME has the advantage of

avoidance of organic solvents, hence yielding a highly pure, conserved extract with minimal artifacts [40].

**Table 1. Percent concentration (%) of the volatile components identified in the EOs of *E. uniflora* L. and *E. supraaxillaris* Spring ex Mart. leaves extracted using hydro-distillation (HD), and headspace (HS) solid-phase micro-extraction (HS-SPME)**

| No. | $RI_{exp}$ [a] | $RI_{lit}$ [b] | Identified Components | MF | E. uniflora | | E. supraaxillaris | |
|---|---|---|---|---|---|---|---|---|
| | | | | | HD | HS-SPME | HD | HS-SPME |
| **1** | 799 | 800 | Octane | $C_8H_{18}$ | 1.33 | - | - | - |
| **2** | 823 | 821 | E-2-Hexenal | $C_6H_{10}O$ | - | 93.87 | - | 46.68 |
| **3** | 840 | 840 | E-3-Hexenol | $C_6H_{12}O$ | - | 0.31 | - | - |
| **4** | 863 | 864 | Isononane | $C_9H_{20}$ | - | - | 0.11 | - |
| **5** | 899 | 900 | n-Nonane | $C_9H_{20}$ | 1.18 | - | 0.36 | - |
| **6** | 928 | 928 | α-Pinene | $C_{10}H_{16}$ | - | - | 8.02 | 28.41 |
| **7** | 987 | 987 | β-Pinene | $C_{10}H_{16}$ | - | 0.29 | 0.44 | 0.41 |
| **8** | 987 | 987 | β-Myrcene | $C_{10}H_{16}$ | - | - | 0.33 | - |
| **9** | 1000 | 1000 | α-phellanderene | $C_{10}H_{16}$ | - | 0.27 | - | - |
| **10** | 1007 | 1000 | n-Decane | $C_{10}H_{22}$ | - | - | 0.07 | - |
| **11** | 1015 | 1015 | o-Cymene | $C_{10}H_{14}$ | - | - | 0.26 | 1.05 |
| **12** | 1025 | 1025 | D-Limonene | $C_{10}H_{16}$ | - | - | 10.31 | 18.64 |
| **13** | 1033 | 1032 | δ-3-carene | $C_{10}H_{16}$ | - | 0.53 | 0.35 | 0.49 |
| **14** | 1036 | 1037 | β-Ocimene | $C_{10}H_{16}$ | 0.63 | 2.05 | 0.10 | 2.07 |
| **15** | 1053 | 1053 | γ-Terpinene | $C_{10}H_{16}$ | - | 0.20 | - | - |
| **16** | 1082 | 1082 | α-Terpinolene | $C_{10}H_{16}$ | - | 0.15 | - | - |
| **17** | 1328 | 1328 | δ-EIemene | $C_{15}H_{24}$ | 0.61 | - | - | - |
| **18** | 1341 | 1341 | Citronellol acetate | $C_{12}H_{22}O_2$ | - | - | 0.14 | - |
| **19** | 1351 | 1351 | α-Cubebene | $C_{15}H_{24}$ | - | - | 0.27 | - |
| **20** | 1376 | 1376 | α-Copaene | $C_{15}H_{24}$ | - | - | 1.53 | -- |
| **21** | 1383 | 1383 | β-Bourbonene | $C_{15}H_{24}$ | - | - | 0.13 | - |
| **22** | 1388 | 1388 | β-Elemene | $C_{15}H_{24}$ | 2.25 | - | 0.53 | - |
| **23** | 1417 | 1417 | β-Caryophyllene | $C_{15}H_{24}$ | 1.10 | 0.22 | 9.87 | 0.65 |
| **24** | 1420 | 1420 | (-)-γ-Elemene | $C_{15}H_{24}$ | 0.52 | - | - | - |
| **25** | 1426 | 1426 | β-Copaene | $C_{15}H_{24}$ | - | - | 0.61 | - |
| **26** | 1446 | 1446 | Muurola-3,5-diene | $C_{15}H_{24}$ | - | - | 0.19 | - |
| **27** | 1451 | 1451 | α-Caryophyllene | $C_{15}H_{24}$ | - | - | 11.82 | - |
| **28** | 1458 | 1458 | β-Gurjunene | $C_{15}H_{24}$ | - | - | 0.36 | - |
| **29** | 1473 | 1473 | γ-Muurolene | $C_{15}H_{24}$ | - | - | 1.46 | - |
| **30** | 1475 | 1470 | Curzerene | $C_{15}H_{20}O$ | 39.85 | - | - | - |
| **31** | 1478 | 1478 | Germacrene D | $C_{15}H_{24}$ | 2.15 | 0.30 | 16.86 | 1.59 |
| **32** | 1482 | 1482 | *β*-Selinene | $C_{15}H_{24}$ | - | - | 0.40 | - |
| **33** | 1486 | 1484 | Muurola-4,5-diene | $C_{15}H_{24}$ | - | - | 0.34 | - |
| **34** | 1489 | 1489 | *δ*-Guaiene | $C_{15}H_{24}$ | 20.91 | 0.60 | - | - |
| **35** | 1489 | 1491 | *γ*-Amorphene | $C_{15}H_{24}$ | - | - | 0.54 | - |
| **36** | 1492 | 1492 | *β*-Cyclogermacrane | $C_{15}H_{24}$ | 6.43 | 0.71 | 2.43 | - |
| **37** | 1496 | 1496 | *α*-Muurolene | $C_{15}H_{24}$ | - | - | 1.14 | - |
| **38** | 1502 | 1502 | *δ*-Cadinene | $C_{15}H_{24}$ | - | - | 0.45 | - |
| **39** | 1507 | 1507 | *γ*-Cadinene | $C_{15}H_{24}$ | - | - | 1.22 | - |
| **40** | 1517 | 1518 | *β*-Cadinene | $C_{15}H_{24}$ | - | - | 4.63 | - |
| **41** | 1525 | 1526 | Cubenene | $C_{15}H_{24}$ | - | - | 0.29 | - |
| **42** | 1531 | 1533 | *α*-Cadinene | $C_{15}H_{24}$ | - | - | 0.46 | - |
| **43** | 1538 | 1538 | Selina-3,7(11)-diene | $C_{15}H_{24}$ | - | - | 0.19 | - |
| **44** | 1549 | 1549 | Germacrene B | $C_{15}H_{24}$ | 6.55 | 0.34 | 0.83 | - |
| **45** | 1562 | 1560 | (-)-Globulol | $C_{15}H_{26}O$ | 1.71 | - | 0.53 | - |
| **46** | 1567 | 1567 | *β*-Caryophyllene oxide | $C_{15}H_{24}O$ | - | - | 0.95 | - |
| **47** | 1569 | 1569 | Viridiflorol | $C_{15}H_{26}O$ | 1.35 | - | - | - |
| **48** | 1571 | 1571 | Ledol | $C_{15}H_{26}O$ | - | - | 0.52 | - |
| **49** | 1572 | 1580 | *β*-Elemenone | $C_{15}H_{22}O$ | 0.92 | - | - | - |
| **50** | 1580 | 1575 | Humulene epoxide I | $C_{15}H_{24}O$ | - | - | 0.39 | - |
| **51** | 1590 | 1590 | Humulene epoxide II | $C_{15}H_{24}O$ | - | - | 1.36 | - |
| **52** | 1600 | 1601 | Epicubenol | $C_{15}H_{26}O$ | - | - | 0.37 | - |
| **53** | 1604 | 1605 | Junenol | $C_{15}H_{26}O$ | - | - | 0.64 | - |
| **54** | 1613 | 1613 | Diepi-Cubenol | $C_{15}H_{26}O$ | - | - | 1.90 | - |
| **55** | 1624 | 1623 | *τ*-Muurolol | $C_{15}H_{26}O$ | - | - | 5.44 | - |
| **56** | 1625 | 1627 | Neointermedeol | $C_{15}H_{26}O$ | 0.96 | - | - | - |
| **57** | 1627 | 1621 | Atractylone | $C_{15}H_{20}O$ | 1.21 | - | - | - |
| **58** | 1637 | 1637 | *α*-Cadinol | $C_{15}H_{26}O$ | - | - | 4.92 | - |
| **Total identified components** | | | | | **89.66** | **99.84** | **94.06** | **99.99** |
| **Oxygenated Components** | | | | | **46.00** | **94.18** | **17.16** | **46.68** |
| **Monoterpenes** | | | | | - | - | - | - |

| | | | | |
|---|---|---|---|---|
| **Sesquiterpenes** | 46.00 | - | 17.02 | - |
| **Miscellaneous** | - | 94.18 | 0.14 | 46.68 |
| **Non-oxygenated components** | **43.66** | **5.66** | **76.90** | **53.31** |
| **Monoterpenes** | 0.63 | 3.49 | 19.88 | 51.07 |
| **Sesquiterpenes** | 40.55 | 2.17 | 56.55 | 2.24 |
| **Miscellaneous** | 2.51 | - | 0.47 | - |

MF: Molecular formula, $RI_{Exp}$: experimental refractive index, $RI_{Lit}$: reference refractive index

**Table 2. Identified hydrocarbons and sterols in the unsaponifiable matter (USM) fraction obtained from *E. uniflora* L. and *E. supraaxillaris* Spring ex Mart. leaves**

| No. | Identified Components | MF | E. uniflora | | | E. supraaxillaris | | |
|---|---|---|---|---|---|---|---|---|
| | | | RT | RRT | % Area | RT | RRT | % Area |
| **1** | Tridecane | $C_{13}H_{28}$ | 8.54 | 0.58 | 0.14 | - | - | - |
| **2** | Tetradecane | $C_{14}H_{30}$ | 10.73 | 0.73 | 6.11 | 9.99 | 0.37 | 0.24 |
| **3** | Pentadecane | $C_{15}H_{32}$ | 11.53 | 0.79 | 4.57 | 11.39 | 0.42 | 0.18 |
| **4** | Hexadecane | $C_{16}H_{34}$ | 12.03 | 0.82 | 18.22 | - | - | - |
| **5** | Heptadecane | $C_{17}H_{36}$ | 13.00 | 0.89 | 20.37 | 13.13 | 0.49 | 0.39 |
| **6** | Octadecane* | $C_{18}H_{38}$ | **14.67** | **1** | **24.38** | 14.02 | 0.52 | 0.11 |
| **7** | Nonadecane | $C_{19}H_{40}$ | 15.57 | 1.06 | 9.98 | 15.62 | 0.58 | 0.60 |
| **8** | Eicosane | $C_{20}H_{42}$ | 16.52 | 1.13 | 3.52 | 16.51 | 0.61 | 9.50 |
| **9** | Henicosane | $C_{21}H_{44}$ | 17.83 | 1.22 | 0.48 | 17.58 | 0.65 | 3.93 |
| **10** | Docosane | $C_{22}H_{46}$ | 18.60 | 1.27 | 6.39 | 18.52 | 0.68 | 17.86 |
| **11** | Tricosane | $C_{23}H_{48}$ | - | - | - | 20.02 | 0.74 | 2.69 |
| **12** | Tetracosane | $C_{24}H_{50}$ | 20.64 | 1.41 | 0.28 | 20.77 | 0.77 | 0.79 |
| **13** | Pentacosane | $C_{25}H_{52}$ | 22.20 | 1.51 | 0.22 | 22.18 | 0.82 | 0.28 |
| **14** | Hexacosane | $C_{26}H_{54}$ | - | - | - | 23.45 | 0.87 | 0.14 |
| **15** | Heptacosane | $C_{27}H_{56}$ | - | - | - | 24.60 | 0.91 | 2.12 |
| **16** | Squalene | $C_{30}H_{50}$ | 25.73 | 1.75 | 0.60 | 25.21 | 0.93 | 7.71 |
| **17** | Stigmasterol** | $C_{29}H_{48}O$ | 26.87 | 1.83 | 3.45 | **27.04** | **1.00** | **33.52** |
| **18** | β-Sitosterol | $C_{29}H_{50}O$ | 29.04 | 1.98 | 0.63 | 29.09 | 1.08 | 8.44 |
| **Percentage of hydrocarbons** | | | | 95.26% | | | 45.75% | |
| **Percentage of phytosterols** | | | | 4.08% | | | 41.96% | |
| **Percentage of total identified compounds** | | | | 99.34% | | | 87.71% | |

RT: Retention time, RRT: Relative Retention time to octadecane, *RRT to Octadecane, **RRT to stigmasterol

For instance, thirteen components were identified in *E. uniflora* accounting for 98.84 % of the total identified volatiles which comprises an oxygenated, aldehyde component signified totally as (*E*)-2-hexenal (93.87%). Moreover, nine components were recognized in *E. supraaxillaris* representing 99.99% with their major scaffold related to non-oxygenated monoterpenes (51.07%) pinpointed by *α*-pinene (28.41%), followed by D-limonene (18.64%, while (*E*)-2-hexenal was the major identified oxygenated, component (46.68 %).

Concerning the GLC analysis results of the unsaponifiable matter (USM) (**Table 2, Supplementary figures S5** and **S6)**, it revealed the presence of a series of hydrocarbons, constituting 95.26% and 45.75% of the total identified compounds in *E. uniflora* and *E. supraaxillaris*, respectively. Octadecane (24.38%), heptadecane (20.37%), and hexadecane (18.22%) were the most acknowledged hydrocarbons in *E. uniflora*, while docosane (17.86 %), eicosane (9.50 %), and squalene (7.71%) were the major tracked in *E. supraaxillaris*. It was obvious that hydrocarbons were more dominant (95.26%) than phytosterols (4.08%) in *E. uniflora*, while being in comparable percentage (45.75% and 41.96%, respectively) in *E. supraaxillaris*. Stigmasterol was the main sterol in both species but with variable concentration. It is identified in *E. supraaxillaris* by 33.52 % which is almost ten-fold more than its percentage in *E. uniflora* (3.45%). In addition, the analysis of the derivatized fatty acids-rich fraction (SM) (**Table 3, Supplementary figures S7** and **S8)** showed the prominence of saturated fatty acids in both species noticed as being three folds more than the unsaturated acids (**Table 3**). For *E. uniflora*, lauric (13.74%) and myristic (12.37%) were the major identified saturated fatty acids, while oleic (4.26%) was the major identified unsaturated fatty acid. On the other hand, lauric (17.76%) and capric (15.94%) were the chief identified saturated fatty acids in *E. supraaxillaris*, while oleic (3.57%) was the major identified unsaturated fatty acid.

Concerning the biological significance of the hydro-distilled EOs and lipoidal matters (LM), samples were comparatively screened for their anti-inflammatory and antimicrobial effects. The anti-inflammatory effect was determined in terms of the ability of the tested samples to inhibit the enzymatic potential of the cyclooxygenase-isoforms COX-1/2 and 5-lipooxygenase (5-LOX). The forementioned enzymes are well known in mediating the inflammatory pathway *via* releasing leukotrienes and prostaglandins down-stream mediators, hence switched on the inflammation cascade[3]. Our screening results showed that, all tested samples exhibited significant inhibition for the three enzymes in dose dependent manner (**Supplementary figures S9-S11)**.

Interestingly EO and LM of *E. supraaxillaris* (ES) exhibited superior activity than its counterpart species (*E. uniflora*). For instance, it inhibited 5-LOX inflammatory activity with $IC_{50}$=4.5±0.5 (EO) and 15.5±0.2 (LM) in comparison to the reference standard, Zileuton (40.0±0.5, **Figure 1, Table 4**).

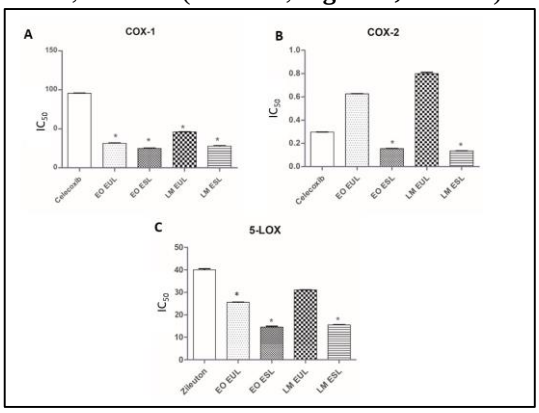


**Figure 1. $IC_{50}$ ±SD of the essential oils (EOs) and lipoidal matters (LM) from both plants on the inflammatory marker, A) COX-1, B) COX-2, and C) 5-LOX. Each value is expressed as the mean of three independent experiments (n=3) and compared to a reference standard drug.**

Moreover, *E. supraaxillaris* samples exhibited selectivity indices (SI) calculated as 161.3 (EO) and 207.4 (LM), revealing their selectivity to COX-2 inhibition rather than COX-1, while the readings were almost near to the SI of celecoxib (SI =322.2, **Table 4)**. The promising anti-inflammatory effect of *E. supraaxillaris* LM sample, may be at least in part, to the synergistic effect of its major fatty acids as capric, lauric, and myristic, in addition to the high phytosterols content (41.96%). It is worth noted that the anti-inflammatory effects induced by plant sterols/stanols have been well demonstrated in many *in vitro* and *in vivo* studies [41]. Reportedly, myristic acid exhibited anti-inflammatory potency especially for skin inflammation [42]. Reis and Co-workers [43] documented the anti-inflammatory activity of lauric acid through the inhibition of eicosanoid pathway and substantially decreasing effect on the level of inflammatory markers induced *in vivo* [44]. Moreover, capric acid displayed significant anti-inflammatory activity against acne-induced infection [45]. Meanwhile, several reports augmented the anti-inflammatory potential of *E. supraaxillaris* volatile constituents just as germacrene D, caryophyllene, and limonene. For instance, previous outcomes showed the wound-healing possibility of caryophyllene local preparation in rat skin injuries, caused by anti-inflammatory, antioxidant, and re-epithelialization mechanisms [46]. D-limonene exhibited anti-inflammatory properties in an ulcerative colitis rat model and in case of bronchial asthma [47,48]. In all, the EOs and LMs, especially ES, exerted their anti-inflammatory mechanism, at least in part, through the dual inhibition of the COX-2 and 5-LOX pathways. Hence, promoting *E. supraxaallaris* as valuable source of potent anti-inflammatory hits with lower side effects.

Nowadays there is an increasing attention in investigating and developing novel antimicrobial agents from different origins to conflict microbial resistance [5]. Thus, we paid great consideration to the antimicrobial screening of the extracted EOs and LMs against selected bacterial and fungal strains using agar well diffusion and microbroth dilution assays. The antimicrobial mechanism varies according to the EO composition as well as the microorganism strain. The agar diffusion assay results (**Table 5)** showed that all gram-positive bacteria being susceptible to all tested treatments, while being resistant to the EO of *E. uniflora* even at the maximum dose. For instance, *E. supraaxillaris* LM displayed the largest zone of inhibition (20.0 mm) at 200 mg/mL, followed by *E. uniflora* LM (18.0 mm, 200 mg/mL). The observed equivalent activity may be correlated to the high lauric acid content detected in LM of both species, which is being 17.76% (ES) and 13.74 (EU). Previous data has highlighted the potent inhibitory potential of the saturated fatty acid, lauric especially against gram-positive bacteria [49]. Meanwhile, we observed that *E. supraaxillaris* EO displayed moderate zones of inhibition (13.0 mm) especially on *S. faecalis* and *E. faecalis* at the tested conc. 20 µL/mL. The forementioned results almost coincide with the reported data about *β*-caryophyllene, one of the paramount constituents in *E. supraaxillaris* EO, as selective antibacterial agent against Gram-positive just as *S. aureus* and *E. faecalis* [50,51]. Interestingly, all tested samples exhibited significant inhibition on Gram-negative bacteria with both species LMs being more efficient than their derived EOs. The largest recorded zones of inhibition (17.0-18.0 mm) were comparatively shared by *E. supraaxillaris* and *E. uniflora* LMs at their maximum tested dose (200 mg/mL), while their EOs displayed moderate zone of inhibition (12.0 mm) especially on *P. aeruginosa* at 20 µL/mL. Gram-positive bacteria are more affected by Eos than Gram-negative bacteria [52] because Gram-negative bacteria have a rigid external membrane which is more complicated and contains a high amount of lipopolysaccharide, so it inhibits the transmission of the hydrophobic constituents into it. However, this extra complicated membrane is not present in Gram-positive bacteria, which are on the contrary enclosed by a dense peptidoglycan membrane not thick enough to repel small antimicrobial molecules, enabling the entree to the cell wall [53]. Furthermore, Gram-positive bacteria may facilitate the penetration of hydrophobic constituents because of the lipophilic edges of lipoteichoic acid found in cell wall [54]. That is to rationale the prominent antimicrobial activity of *E. supraaxillaris* EO (rich with hydrophobic volatiles) on Gram-positive, while *E. uniflora* displayed obvious

activity on Gram-negative (rich with polar oxygenated volatiles). Lastly, all tested samples showed good antifungal activity with their EOs displayed the best measured zones of inhibition (30.0-31.0 mm), while their LMs exhibited moderate zones of inhibition (8.0-17.0 mm) which may be correlated to the prominence of lauric acid as mentioned before. Previous data highlighted the possibility of using lauric acid as another therapy for antibiotic remedy of *Acne vulgaris* [55], while Akula and his research team [56] documented its activity against *Candida albican.* On the other side, the dose response effect of all tested samples in the broth microdilution assay was presented as **Supplementary figures S12-S15,** while the calculated MICs were delineated in **Table 6.** It showed that both species' EOs possessed efficient antimicrobial potential with MICs >40 µL/mL, which is almost 5-10 folds potent than their corresponding LM (**Table 6**).

**Table 3. Identified fatty acids in the saponifiable matter (SM) fraction obtained from *E. uniflora* L. and *E. supraaxillaris* Spring ex Mart. leaves**

| No. | Compound | MF | Saturation degree | E. uniflora | | | E. supraaxillaris | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | RT | RRT | % Area | RT | RRT | % Area |
| **1** | Caprylic acid | $C_8H_{16}O_2$ | C8:0 | 8.63 | 0.84 | 1.70 | 8.52 | 0.83 | 4.33 |
| **2** | Capric acid | $C_{10}H_{20}O_2$ | C10:0 | 9.43 | 0.92 | 10.25 | 9.47 | 0.92 | 15.94 |
| **3** | Undecylic acid | $C_{11}H_{22}O_2$ | C11:0 | 9.98 | 0.97 | 10.57 | 10.09 | 0.98 | 14.86 |
| **4** | **Lauric acid*** | **$C_{12}H_{24}O_2$** | **C12:0** | **10.28** | **1.00** | **13.74** | **10.32** | **1.00** | **17.76** |
| **5** | Myristic acid | $C_{14}H_{28}O_2$ | C14:0 | 11.09 | 1.08 | 12.37 | 11.14 | 1.08 | 14.23 |
| **6** | Pentadecylic acid | $C_{15}H_{30}O_2$ | C15:0 | 11.86 | 1.15 | 8.83 | 11.91 | 1.15 | 7.45 |
| **7** | Palmitic acid | $C_{16}H_{32}O_2$ | C16:0 | 12.68 | 1.23 | 4.90 | 12.69 | 1.23 | 1.50 |
| **8** | Stearic acid | $C_{18}H_{36}O_2$ | C18:0 | 14.05 | 1.37 | 7.36 | - | - | - |
| **9** | Oleic acid | $C_{18}H_{34}O_2$ | C18:1 | 14.34 | 1.39 | 4.26 | 14.35 | 1.39 | 3.57 |
| **10** | Linoleic acid | $C_{18}H_{32}O_2$ | C18:2 | 14.84 | 1.44 | 3.28 | 14.85 | 1.44 | 1.44 |
| **11** | Linolenic acid | $C_{18}H_{30}O_2$ | C18:3 | 15.44 | 1.50 | 2.97 | 15.45 | 1.50 | 0.78 |
| **12** | Arachidic acid | $C_{20}H_{40}O_2$ | C20:0 | 16.33 | 1.59 | 5.25 | 16.39 | 1.59 | 1.28 |
| **13** | Eicosenoic acid | $C_{20}H_{38}O_2$ | C20:1 | 16.92 | 1.65 | 0.99 | 17.09 | 1.66 | 0.51 |
| **14** | Arachidonic acid | $C_{20}H_{32}O_2$ | C20:4 | 17.89 | 1.74 | 1.37 | 18.04 | 1.75 | 1.53 |
| **15** | Behenic acid | $C_{22}H_{44}O_2$ | C22:0 | - | - | - | 20.12 | 1.95 | 0.44 |
| **16** | Erucic acid | $C_{22}H_{42}O_2$ | C22:1 | - | - | - | 20.59 | 2.00 | 0.02 |
| **17** | Lignoceric Acid | $C_{24}H_{48}O_2$ | C24:0 | 24.15 | 2.35 | 0.32 | 24.16 | 2.34 | 0.01 |
| **18** | Nervonic acid | $C_{24}H_{46}O_2$ | C24:1 | 24.52 | 2.39 | 0.78 | 24.54 | 2.38 | 0.21 |
| **Percentage of saturated fatty acids** | | | | | | 75.29% | | | 77.80% |
| **Percentage of unsaturated fatty acids** | | | | | | 13.65% | | | 8.06% |
| **Percentage of total identified compounds** | | | | | | 88.94% | | | 85.86% |

RT: Retention time, RRT: Relative retention time, *RRT to lauric acid

**Table 4: Anti-inflammatory effect (represented by $IC_{50}$ ±SD) of the extracted essential oils (EOs) and lipoidal matter (LM) from *E. uniflora* L. *and E. supraaxillaris* Spring ex Mart. leaves in 5-LOX, COX-1, and COX-2 enzyme-based assays**

| Tested Sample | $IC_{50}$ ±SD | | | |
|---|---|---|---|---|
| | 5-LOX | COX-1 | COX-2 | SI* |
| ***E. uniflora* HD EO (µL/mL)** | 25.5±0.2 | 31.5±0.2 | 0.625±0.001 | 50.4 |
| ***E. supraaxillaris* HD EO (µL/mL)** | 14.5±0.5 | 25.0±0.2 | 0.155±0.001 | 161.3 |
| ***E. uniflora* LM (mg/mL)** | 31.0±0.1 | 46.0±0.1 | 0.800±0.010 | 57.5 |
| ***E. supraaxillaris* LM (mg/mL)** | 15.5±0.2 | 28.0±0.1 | 0.135±0.001 | 207.6 |
| **Zileuton (µg/mL)** | 40.0±0.5 | - | - | - |
| **Celecoxib (µg/mL)** | - | 95.7±0.1 | 0.297±0.001 | 322.2 |

***COX selectivity index which is defined as $IC_{50}$ (COX-1)/$IC_{50}$ (COX-2)**

**Table (5): Measured zones of inhibition (in mm) of the tested essential oils (EOs) and lipoidal matters (LMs) of *E. uniflora* L. (EU) and *E. supraaxillaris* Spring ex Mart. (ES) in agar well diffusion assay against selected, reference microbial strains**

| Tested samples | EU EO* | | | | ES EO* | | | | EU LM** | | | | ES LM** | | | | AK | AX | NOR | OFX | SAM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Tested Conc.** | 2.5 | 5 | 10 | 20 | 2.5 | 5 | 10 | 20 | 50 | 100 | 150 | 200 | 50 | 100 | 150 | 200 | 30 | 25 | 10 | 5 | 20 |
| **Gram (+ve)** | | | | | | | | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **C. perfringens** | - | - | - | - | - | - | - | 10 | 11 | 11 | 13 | 14 | 13 | 14 | 16 | 19 | 23 | 18 | 34 | 30 | 11 |
| **E. faecalis** | - | - | - | - | - | - | 12 | 13 | 12 | 14 | 15 | 18 | 14 | 16 | 17 | 18 | 7 | 7 | 8 | 7 | 7 |
| **S. faecalis** | - | - | - | - | 10 | 11 | 11 | 13 | 12 | 12 | 13 | 16 | 14 | 15 | 17 | 20 | 25 | - | 27 | 26 | - |
| **Gram (-ve)** | | | | | | | | | | | | | | | | | | | | | |
| **K. pneumoniae** | 7 | 7 | 8 | 9 | - | - | - | 8 | 10 | 12 | 15 | 16 | 14 | 15 | 17 | 18 | 21 | 16 | 30 | 30 | 10 |
| **P. aeruginosa** | - | 8 | 10 | 12 | - | - | 11 | 12 | 11 | 12 | 14 | 16 | 14 | 14 | 16 | 18 | 21 | - | 30 | 20 | - |
| **Fungi** | | | | | | | | | | | | | | | | | | | | | |
| **C. albicans** | - | 8 | 9 | 12 | - | 10 | 12 | 15 | 8 | 10 | 11 | 16 | 11 | 13 | 14 | 17 | NT | NT | NT | NT | NT |
| **A. niger** | - | - | 19 | 30 | 11 | 16 | 22 | 31 | 8 | 8 | 10 | 15 | - | - | - | - | NT | NT | NT | NT | NT |

*****Conc. in µl/ml, ** Conc. in mg/mL, NT: not tested

**Table (6): Minimum inhibitory concentrations (MICs) of the tested essential oils (EOs) and lipoidal matters (LMs) of *E. uniflora* L. and E. *supraaxillaris* Spring ex Mart. against selected, reference microbial strains**

| Tested samples | MICs | | | | | | |
|---|---|---|---|---|---|---|---|
| | ***C. perfringens*** | ***E. faecalis*** | ***S. faecalis*** | ***K. pneumoniae*** | ***P. aeruginosa*** | ***C. albicans*** | ***A. Niger*** |
| **EU EO*** | >40 | >40 | >40 | >40 | >40 | >40 | ≥40 |
| **ES EO*** | >40 | >40 | >40 | >40 | >40 | >40 | ≥40 |
| **EU LM**** | ≥200 | ≥200 | ≥200 | ≥400 | ≥400 | ≥400 | ≥400 |
| **ES LM**** | >290 | >290 | ≥290 | >290 | >290 | ≥290 | >290 |

*****Conc. in µl/ml, ** Conc. in mg/mL

## 4. Conclusion

Essential oil's composition differs greatly in terms of plants species. *Eugenia uniflora* and *E. supraaxillaris* might be prospective sources for curzerene and germacrene D, respectively. The variable ratio between oxygenated and non-oxygenated volatiles alters the permeability of bacterial membranes, hence clarify their antimicrobial action and synergistic effect. *E. uniflora* and *E. supraaxillaris* displayed comparable hydrocarbon content. Lipoidal matter rich in phytosterols are correlated to more significant anti-inflammatory dual properties. Essential oils and lipoidal matter are eco-friendly alternatives to synthetic drugs with prosperous future in the treatment of infectious and inflammatory diseases.

## 5. Authors contribution

H.E., R.I., A.K., M. I., conceptualization, investigation, and supervision. H.E., S.M. analyzed the GC and biological data. S.M. extracted and analyzed the lipoidal matter data. All authors write, read, and agreed the last form of the manuscript.

## 6. Conflicts of interest

The authors reported no potential conflict of interest.

## 7. References


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