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Chemical composition and anti-inflammatory activity of the essential oils of *Psidium guajava* fruits and leaves

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Psidium guajava L. (Myrtaceae) has been used traditionally against gastrointestinal disturbances and respiratory ailments. The chemical composition of the essential oil of both leaves and fruits were elucidated by gas–liquid chromatography/mass spectrometry (GLC/MS). Forty-five and forty-two compounds, accounting for 93.7% and 89.7% of the fruit and leaf oil, were identified, respectively. The dominant compounds were β-caryophyllene (17.6%) and limonene (11.0%) for the fruit oil and β-caryophyllene (16.9%) and selin-7(11)-en-4α-ol (8.3%) for the leaf oil. The radical scavenging activities of both essential oils were assessed by the diphenyl picrylhydrazyl (DPPH$^{\bullet}$) and deoxyribose degradation assays. Guava leaf oil reduced DPPH$^{\bullet}$ radicals and prevented the degradation of the deoxyribose with IC_{50} values of 3.59 and 12.64 μg/mL. The *in vitro* cytotoxicity of the oils in HepG2 and MCF-7 carcinoma cells was examined using the SRB assay (IC_{50} 32.53 and 49.76 μg/mL for the leaves and fruit oils against HepG2 cells). Inhibition of 5-lipoxygenase (5-LOX) was used to evaluate the anti-inflammatory activity of both oils (IC_{50} 130.69 and 196.45 μg/mL for the leaves and fruit oils). The anti-inflammatory activity was explained via virtual docking of the major identified compounds to the main sites in the 5-LOX crystal structure.

Keywords: *Psidium guajava*; essential oil; chemical composition; biological activity; cytotoxicity; molecular docking

1. Introduction

Natural products with their diverse biological and pharmacological activities represent a gold mine for scientists searching for lead compounds for the treatment of health disorders and infections. In the last few years, with an increasing interest in aromatherapy, emphasis has been mainly on the anti-inflammatory and antioxidant properties of essential oils (1, 2).

Based on traditional Chinese and Ayurvedic usage, plants belonging to the family Myrtaceae are well known as rich sources of valuable essential oils such as clove oil, tea tree oil, cajuput oil, pimento and *Eucalyptus* oil. Most of these oils are of medicinal relevance in alleviating the symptoms associated with nasopharyngeal infections, cough and nasal congestion (3, 4). The genus *Psidium* (Myrtaceae) comprises around 70 species distributed in the American and Asian tropics. *Psidium guajava* L., commonly known as guava, is an evergreen tree, which is grown in many tropical and subtropical areas of the world, including Egypt (5, 6).

Guava has been widely used in Central and South America, West and North Africa and some parts of South-East Asia to treat gastrointestinal disturbances, such as dysentery, and stomach pain, diabetes, fever (malaria) cough, ulcers, boils, wounds, sore throat and laryngitis (7–11). *Psidium* extracts are also employed for antinociceptive, antimicrobial, hepatoprotective and antispasmodic properties (9). Moreover, in Egypt, guava leaf infusion and/or inhaled vapor are widely used for their anti-inflammatory effect in soothing a persistent cough and alleviating the symptoms of the common cold (12).

Several studies have been conducted on the composition of the volatile components of guava fruits and leaves (13–15). The main components of the leaf oil were reported to be α-pinene, β-pinene, 1,8-cineole and β-caryophyllene, while hexanal, 2-hexenal, β-caryophyllene and γ-cadinene constituted the major components in the fruit oil. The antioxidant and anti-inflammatory activities of both guava fruit and leaf extracts were attributed to flavonoids, tannins (up to 12%) and triterpenes (8, 16).

The biological activities of the essential oil of guava fruits and leaves have not been fully investigated. In this study, we determined their antioxidant and anti-inflammatory activities as well as their cytotoxicity in HepG2 and MCF-7 carcinoma cells in an attempt to explain rationally some of the ethnopharmacological uses of guava.

2. Experimental

2.1 Plant material

Both the fruits and leaves of *P. guajava* were collected in September 2011 from fruiting trees by one of the

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authors (S.E.) from local fields located east of Cairo. The identity of the plant was ascertained morphologically by Prof. Abdel Rahman Al-Newaihi, Department of Botany, Faculty of Science, Ain Shams University. Voucher specimens of the plant material are deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, under the number P-330.

2.2 Chemicals

Cell culture media, supplements and dimethyl sulfoxide (DMSO) were purchased from Roth® (Karlsruhe, Germany) and Greiner® Labortechnik (Frickenhausen, Germany). Diphenyl picryl hydrazyl (DPPH), 2-deoxyribose (2-DR), norhydroguaretic acid (NDGA), sodium linoleate, quercetin, and doxorubicin were purchased from Sigma® (Sigma-Aldrich, St. Louis, MO) and Gibco® (Invitrogen; Karlsruhe, Germany). Thiobarbituric acid was purchased from Fluka® (Buchs, Switzerland). Other chemicals, including; EDTA, ferric chloride ($FeCl_3$), trichloroacetic acid, and sulforhodamine B (SRB) were of the highest analytical grade.

2.3 Isolation of the essential oils

Essential oils were obtained by hydrodistillation of fresh leaves and fruits (without the seeds) for 6 hours using a Clevenger-type apparatus; the yields were 0.5% and 0.12% (v/w fresh material), respectively. Both oils were dried over anhydrous sodium sulfate and kept in sealed vials at −30°C for analyses.

2.4 Gas–liquid chromatography

2.4.1 GLC–FID analysis

Gas–liquid chromatography (GLC) analyses were carried out on a Focus GC® (Thermo Fisher Scientific®, Milan, Italy) equipped with TR-5MS fused bonded column (30 m × 0.25 mm × 0.25 μm) (Thermo Fisher Scientific®, Florida) and flame ionization (FID) detector; carrier gas was nitrogen (1.5 mL/min); the operating conditions were: initial temperature 40°C, 1 minute isothermal followed by linear temperature increase until 230°C at a rate of 4°C/minute, then 5 minutes isothermal. Detector and injector temperatures were 300° and 220°C, respectively. The split ratio was 1: 20. Chrom-card® chromatography data system ver. 2.3.3 (Thermo Electron Corp.®, Florida) was used for recording and integrating of the chromatograms. Average areas under the peaks of three independent chromatographic runs were used to calculate the % composition of each component.

2.4.2 GLC–MS analysis

The analyses were carried out on a Focus GC® (Thermo Fisher Scientific®, Milan, Italy) equipped with the same column and conditions mentioned in the GLC–FID. The capillary column was directly coupled to a quadrupole mass spectrometer (MS) Polaris Q (Thermo Electron Corp.®, Milan, Italy). The injector temperature was 220°C. Helium carrier gas flow rate was 1.5 mL/minute. All the mass spectra were recorded with the following condition: filament emission current, 100 mA; electron energy, 70 eV; ion source, 250°C; diluted samples were injected with split mode (split ratio, 1:15). Compounds were identified by comparing their spectral data and retention indices with the Wiley Registry of Mass Spectral Data, 8th edition, NIST Mass Spectral Library (December 2005) and other published data (17).

2.5 Antioxidant activity

2.5.1 Radical scavenging activity

The radical scavenging activity of the essential oils was evaluated using diphenyl picryl hydrazyl ($DPPH^{\bullet}$) as an indicator (18). Equal volumes of sample solutions (in methanol) containing 0.02–40 mg/mL of the oils and 0.2 mM of a methanolic solution of $DPPH^{\bullet}$ were mixed and the absorbance was measured against a blank at 520 nm using a Jenway® 6800 UV/VIS spectrophotometer (Essex) after incubation in the dark for 30 minutes at room temperature compared with $DPPH^{\bullet}$ control after background subtraction. Quercetin was used as a positive control. The percent inhibition was calculated from three different experiments using the following equation: inhibition (%) = $100\times[A_{520}$ (control) − A_{520} (sample)/A_{520} (control)].

2.5.2 Deoxyribose degradation assay

The ability of the samples to prevent the degradation of the deoxyribose was determined after Houghton et al. (19). A mixture of freshly prepared 28 mM 2-deoxy-2-ribose (2-DR) in phosphate buffer pH 7.1, 1.04 mM EDTA and 200 μM $FeCl_3$, 1.0 mM H_2O_2 and 1.0 mM ascorbic acid was mixed with an equal volume of various sample concentrations (0.002–6 mg/mL of each oils in methanol) and kept at 37°C for 1 hour. A mixture of thiobarbituric acid and 2.8% trichloroacetic acid (1:1 v/v) was added to the reaction mixture and incubated at 100°C for 20 minutes. Absorbance was measured at 540 nm against blank using a Jenway® 6800 UV/VIS spectrophotometer (Essex). Quercetin was used as a positive control. Inhibition of deoxyribose degradation was measured in triplicate at 540 nm as above.

2.6 Anti-inflammatory activity – Inhibition of 5-lipoxygenase

Inhibition of the soybean 5-lipoxygenase was determined spectrophotometrically (20). Briefly, 0.1 M

phosphate buffer (1 mL) pH 9.0, containing 10 µl enzyme (7.9 U/mL) and 20 µl of 10 different sample concentrations (10–350 µg/mL oils in ethanol) were incubated at room temperature for 10 minutes. The reaction was initiated by adding 25 µl of 62.5 µM sodium linoleate and the reaction kinetics were monitored at 234 nm at 10-second intervals using a Jenway® 6800 UV/VIS spectrophotometer (Essex). The initial reaction rates were determined from the slope of the straight line portion of the curve and inhibition of the enzyme activity was calculated from three independent experiments by comparison with the control (ethanol). Nordihydroguaiaretic acid (NDGA) was used as a positive control (IC_{50} 0.24 µg/mL).

2.7 Cytotoxicity and cell proliferation assay

HepG2 (hepatic cancer) and MCF-7 (breast cancer) cell lines were maintained in Dulbecco's Modified Eagle Medium complete media (l-glutamine supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin and 100 U/mL streptomycin). Cells were grown at 37°C in a humidified atmosphere of 5% CO_2. All experiments were performed with cells in the logarithmic growth phase.

Sensitivity to drugs was determined in triplicate using the SRB (sulforhodamine B) cell viability assay (21). Exponentially growing HepG2 and MCF-7 cells were collected using 0.25% Trypsin–EDTA and plated in 96-well plates Greiner Labortechnik® (Frickenhausen, Germany) at 1000–2000 cells/well. The cells were cultivated for 24 hours and then incubated with various concentrations of the serially diluted tested samples (stock solution 1 mg/mL) at 37°C for 72 hours and subsequently fixed with TCA (10%) for 1 hour at 4°C. After several washings, cells were exposed to 0.4% SRB solution for 10 minutes in a dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris–HCl was used to dissolve the SRB-stained cells and optical density intensity was measured at 545 nm using a microplate reader Chromate® 4300 (Awareness Technology Inc. Florida).

2.8 Molecular modeling study

The X-ray crystal structure of human 5-lipoxygenase enzyme (PDB ID 3O8Y) was downloaded from protein data bank (www.pdb.org). The structure of the enzyme was established using the default protein preparation protocol of Accelry's discovery studio 2.5 (Accelrys®, Inc., San Diego). First, hydrogen atoms were added and then the protein structure was cleaned from any unwanted interactions. The binding site was detected based on the reported data beside the catalytic domain of the enzyme just near the metal ion Fe (22). Following the binding site determination, the structures of our compounds were docked inside the binding site using 200,000 operations with selection pressure of 1 and niche size of 2 and leaving the other GOLD parameters at their default values. CHARMm force field was assigned and the binding energies were calculated using distance dependent dielectric implicit solvation model for the selected docking poses.

2.9 Statistical analysis

All experiments were carried out three times unless mentioned in the procedure. Continuous variables were presented as mean± SE. The IC_{50} was determined as the drug concentration that resulted in a 50% reduction in cell viability or inhibition of the biological activity. IC_{50} values were calculated using a four-parameter logistic curve (SigmaPlot® 11.0), and all the data were statistically evaluated using Student's *t*-test and/or the Kruskal–Wallis test (GraphPad Prism® 5.01, GraphPad Software, Inc., CA) followed by Dunn's *post-hoc* multiple comparison test when the significance value is <0.05 using the same significance level.

3. Results and discussion

3.1 GLC–FID and GLC–MS analyses

Essential oils of *P. guajava* have a pale yellow color and characteristic pleasant odors. Forty-five and forty-two compounds, accounting for 93.7% and 89.7% of the essential oils from the fruits and leaves, were unambiguously identified. Quantitative and qualitative analytical results are shown in Table 1. β-Caryophyllene (17.6%), limonene (11.0%), β-caryophyllene oxide (6.7%), α-selinene (6.6%) and β-selinene (6.4%) were the dominant compounds in the fruit oil, while the leaf oil consisted of β-caryophyllene (16.9%), 4α-selin-7 (11)-enol (8.3%), β-caryophyllene oxide (6.5%) and α-selinene (6.5%). The presence of a high percentage of limonene (11.0%) in the fruit oil is the base for the characteristic pleasant citrus odor. Although it is common to find diverse profiles of essential oils obtained from different plant parts, a comparison of both guava fruit and leaf oils revealed only some minor quantitative variations (23).

These results are in accordance with previously published data (14, 24, 25). However, cultivars, regional climatic variation, degree of fruits maturity and the method of essential oil extraction have a great impact on the profiles of volatiles. Nevertheless, β-caryophyllene and its oxidized form are always the main component of the oils (26, 27).

3.2 Antioxidant and anti-inflammatory activity

The antioxidant activity of the essential oils was determined via the DPPH and deoxyribose assays. The concentrations that caused a 50% inhibition (IC_{50}) are

Table 1. The chemical composition of the *Psidium guajava* essential oils.

Compound name	Retention index (RI)		Composition[c] (%)		Methods of identification
	Reported	Calculated	Leaves	Fruits	
α-Pinene[a,b]	929	929	0.3	0.4	MS, RI, AT
β -Pinene[a]	979	978	0.2	0.2	MS, RI, AT
δ-2-Carene	998	992	0.1	0.3	MS, RI
α-Phellandrene	1007	1011	0.1	0.1	MS, RI, AT
α-Terpinene[a]	1017	1022	0.1	0.2	MS, RI, AT
p-Cymene[a,b]	1033	1032	0.1	0.5	MS, RI, AT
Limonene[a,b]	1032	1033	0.2	11.0	MS, RI, AT
1,8-Cineole[a,b]	1037	1040	5.4	0.8	MS, RI, AT
cis-β-Ocimene[a,b]	1037	1041	0.7	0.2	MS, RI
trans-β-Ocimene[a,b]	1050	1052	0.2	0.2	MS, RI, AT
γ-Terpinene[a]	1060	1065	0.1	0.1	MS, RI
Terpinolene[a]	1089	1092	0.2	0.3	MS, RI
allo-Ocimene[a]	1132	1135	0.1	–	MS, RI
α-Terpineol[a,b]	1207	1208	0.1	0.1	MS, RI, AT
Carvacrol[a]	1313	1316	0.2	0.5	MS, RI, AT
α-Copaene[a,b]	1377	1380	1.0	2.1	MS, RI
α-Gurjunene	1410	1412	0.4	0.1	MS, RI
β-Caryophyllene[a,b]	1428	1428	16.9	17.6	MS, RI, AT
β-Copaene	1432	1435	1.7	2.1	MS, RI
β-Gurjunene	1434	1438	–	0.2	MS, RI
Aromadendrene[a,b]	1445	1447	2.8	2.5	MS, RI
allo-Aromadendrene	1460	1456	–	0.8	MS, RI
α-Humulene[a,b]	1466	1466	1.0	1.0	MS, RI
γ-Gurjunene	1460	1470	0.5	0.8	MS, RI
Chamigrene[a]	1478	1482	0.5	–	MS, RI
Muuroladiene[a]	1481	1484	0.5	0.3	MS, RI
Germacrene D[a]	1485	1492	0.3	0.7	MS, RI
β-Selinene[a,b]	1495	1501	6.3	6.4	MS, RI
α-Selinene[a,b]	1505	1508	6.5	6.6	MS, RI
Valencene	1513	1516	0.6	0.8	MS, RI
δ-Cadinene[a,b]	1528	1528	5.3	4.9	MS, RI
α-Calamenene	1540	1535	2.4	1.0	MS, RI
α-Calacorene[a]	1546	1544	1.6	0.9	MS, RI
Germacrene B[a]	1560	1569	0.4	0.4	MS, RI
Viridiflorol[a]	1580	1576	1.7	2.2	MS, RI
Spathulenol[a]	1591	1591	2.3	2.1	MS, RI
β-Caryophyllene-oxide[a,b]	1606	1596	6.5	6.7	MS, RI
cis-Isolongifolanone	1613	1619	–	1.5	MS, RI
β-Eudesmol[b]	1624	1622	–	3.7	MS, RI
Daucol	1640	1642	4.8	4.6	MS, RI
Cedr-8(15)-en-9-ol		1651	2.0	1.9	MS
Cubenol[a]	1646	1655	4.4	1.8	MS, RI
τ-Cadinol[a,b]	1652	1658	–	2.5	MS, RI
δ-Cadinol[a,b]	1658	1663	1.8	0.8	MS, RI
Cadalene	1671	1673	0.2	1.2	MS, RI
Selin-7(11)-en-4-α-ol[a]	1675	1678	8.3	0.5	MS, RI
α-Santalol	1683	1689	0.9	0.1	MS, RI
Total			89.7	93.7	

Notes: [a]Compounds previously reported in leaves. [b]Compounds previously reported in fruits. [c]Average of three analyses. MS, identification based on mass spectral data; RI, identification based on comparison with published retention indices in Wiley Registry of Mass Spectral Data, 8th edition, NIST Mass Spectral Library (December 2005) and other published data (17); AT, identification based on co-chromatography with authentic samples. Specific gravity of both leaf and fruit oils at 25°C are 0.8907 and 0.9023, respectively.

documented in Table 2. Leaves and fruit oils showed an IC_{50} of 3.59 and 8.11 mg/mL in DPPH and an IC_{50} of 12.64 and 42.78 μg/mL, in the desoxyribose assay, respectively. Whereas tannins and other antioxidant phenolics dominate the non-volatile part of guava leaves (3), no phenolic constituents could be traced in both oils. The presence of a high concentration of oxygenated monoterpenes and sesquiterpenes in both oils are relevant for the observed antioxidant activities, especially in the desoxyribose assay (28–30). Thus, the essential oils are an additional source of antioxidants (besides polyphenols) in guava leaves (16, 31).

Table 2. Antioxidant activities of essential oils from fruits and leaves of *Psidium guajava*: IC_{50} values of scavenging diphenyl picrylhydrazyl (DPPH$^{\bullet}$) radicals, prevention of the of 2-deoxyribose (2-DR) degradation and inhibition of 5-lipoxygenase.

	DPPH$^{\bullet}$ assay	2-DR assay	5-Lipoxygenase
Guava fruit oil	8.11±0.21 mg/mL	42.78±7.01 µg/mL	49.76±5.23 µg/mL
Guava leaf oil	3.59±0.06 mg/mL	12.64±2.13 µg/mL	32.53±6.11 µg/mL
Positive control[a]	3.78±0.13 µg/mL	5.18±1.77 µg/mL	0.24±0.17 µg/mL

Notes: [a]Quercetin for DPPH and 2-DR assays, nordihydroguaiaretic acid for 5-LOX assay. The results are presented as mean ± SD of three independent replicates.

The essential oils of leaves and fruits inhibit soybean 5-LOX (Table 2) with IC_{50} values of 32.53 and 49.76 µg/mL, respectively, indicating a relevant anti-inflammatory activity as compared with other essential oils measured with the same technique (20, 32). This inhibition activity may be attributed to the presence of β-caryophyllene, limonene and β-caryophyllene oxide, known 5-LOX inhibitors (33). Generally, inflammation involves the formation of both prostaglandins and leukotrienes as mediators followed by the liberation of neutrophils and the production of the reactive oxygen species (ROS). The inhibition of COX-II is normally used as a criterion for anti-inflammatory activity. This will lead to the dismissal of other potential lead compounds, which might serve as good inhibitors of 5-LOX and other inflammatory mediators as NFκB, ROS and many others (34).

3.3 Docking study

Molecular docking was performed in order to evaluate a possible binding mode of the major 5-LOX inhibitors, such as β-caryophyllene, β-caryophyllene oxide and limonene to the active site of human 5-lipoxygenase enzyme. It has been previously reported (35) that the free binding energy of the inhibitors is directly correlated with the inhibitory concentration. Due to the hydrophobicity and lower functionality of the major compounds, we have selected the free binding energy of these compounds as the comparison factor in docking experiments. The free binding energies of our docked compounds and the known 5-LOX inhibitor nordihydroguaiaretic acid (NDGA) are shown in Table 3. Inspection of the active site of 5-LOX revealed many hydrophobic residues such as Leu 414 and Phe 421 and many others. The binding mode of β-caryophyllene oxide is shown in Figure 1, where the Leu 414 and Phe 421 were involved in hydrophobic interactions. The binding energy revealed that β-caryophyllene oxide had the best binding affinity (−135.143 kcal/mol) while limonene has the least binding affinity (−88.316 kcal/mol) indicating a lower inhibitory activity of 5-LOX.

3.4 Cytotoxic activity

The safety of the essential oils in humans is of concern if they are applied orally. As a pilot study the cytotoxicity of the essential oils were evaluated using two human cancer cell lines (HepG2 and MCF-7). The cytotoxicity of the essential oils after 72 hours incubation are illustrated in Figure 2, with IC_{50} values ranging between 130.69 and 351.00 µg/mL for guava leaf oil and 196.45 and 544.38 µg/mL for guava fruit oil. The cytotoxicity was much lower in MCF-7 cells than in HepG2 cells. Guava fruits with an essential oil

Table 3. Molecular docking experiments: Binding of the major oil components to human 5-lipoxygenase (5-LOX) indicated by binding energy values ($\Delta G_{binding}$).

Compound name	Binding energy ($\Delta G_{binding}$)
Limonene	−88.316
β-Caryophyllene	−130.538
β-Selinene	−104.257
α-Selinene	−108.025
β-Caryophyllene-oxide	−135.143
Selin-7(11)-en-4-α-ol	−128.198
Nordihydroguaiaretic acid	−74.62

Notes: The ligand–enzyme interaction energy value ($\Delta G_{binding}$) was calculated using the following equation: $\Delta G_{binding} = E_{complex} - (E_{5\text{-}LOX} + E_{ligand})$, where $E_{complex}$ was the potential energy for the complex of 5-LOX bound with the ligand, $E_{5\text{-}LOX}$ was the potential energy of the enzyme alone and E_{ligand} was the potential energy for the ligand alone.

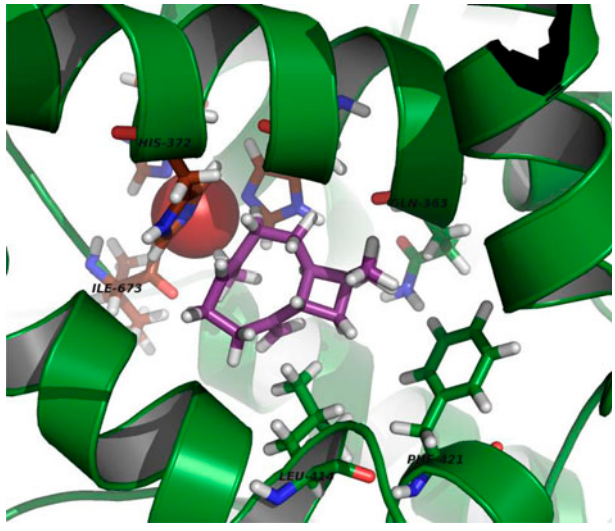

Figure 1. Binding mode for β-caryophyllene in the active site of human 5-lipoxygenase.

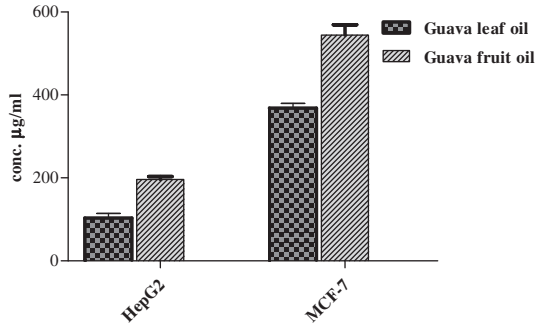

Figure 2. Cytotoxicity: IC_{50} values of essential oils from leaves and fruits in HepG2 and MCF-7 cell lines.

concentration of 0.11% have been consumed without any negative results, indicating that the essential oils are not relevant from a toxicological point of view.

In experiments with human mouth epidermal carcinoma (KB) and murine leukaemia (P388) cells, a 10-fold higher cytotoxicity had been reported for essential oils of guava leaves (36). The cytotoxicity of the essential oils is mediated through the lipophilic terpenoids, which probably influence the integrity of the biomembranes and membrane proteins (37).

4. Conclusion

The increasing interest in the last decade in aromatherapy is based on essential oils, which are composed of diverse volatile plant secondary metabolites with multiple biological activities. Although essential oils are widely used in therapy, limited scientific data are available to validate and clarify the possible mechanisms by which these oils exert most of their claimed activities. To our knowledge, this is the first study on the biological activity of essential oils from guava fruits and leaves. The investigated oils are isolated from a popular edible fruit and palatable leaves, both could serve as convenient and effective nutraceuticals. The significant radical scavenging activity of oils and the substantial inhibition of lipoxygenase by both oils can rationally explain their pharmacological use in the form of inhalation to improve several upper respiratory tract ailments associated with inflammation. However, further *in vivo* studies with animals and humans are required for thorough investigation of the anti-inflammatory activity.

Declaration of interest

The authors declare that they have no conflicts of interest to disclose.

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