Original Article

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Valorization of the peel and husk of guaraná (*Paullinia cupana*): oil extraction, chemical composition, cytotoxicity, and antitumor potential

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Abstract: Guaraná (*Paullinia cupana*) is a fruit native to the Amazon region. Due to its widespread use, primarily in beverages, its production has grown over the years. The seed is the only commercially valuable part of the fruit so the guaraná production chain generates byproducts, including peel, husk,

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and spent seed. Both the husk and peel can be used as raw materials for oil extraction. The use of new oil sources is important for the food and pharmaceutical industries, as their constituents can be used in various applications. This study aimed to extract oil from guaraná byproducts (peel and husk) using Soxhlet extraction, characterize it by gas chromatography–mass spectrometry (GC–MS), and evaluate its biological potential through cytotoxicity and antitumor assays. The peel oil mainly contained the compound 8-androsten-3-ol, 17-(2-methylallyl)-4,4,14-trimethyl, followed by cycloartenol and (E)-14 α-methyl-5 α-ergosta-8,23-dien-3 β-ol. In husk oil, the main compounds were 7-octadecenoic acid methyl ester and palmitic acid methyl ester. In L929 cells, peel oil showed cytotoxicity at concentrations above 250 μg mL^{-1}, and husk oil at concentrations above 100 μg mL^{-1}. Regarding antitumor activity, at concentrations where the oils did not exhibit cytotoxicity, husk oil reduced metabolic activity by up to 21% and peel oil by up to 67% in C6 cells. For A549 cells, metabolic activity decreased to approximately 65% for peel oil and 76% for husk oil; both oils showed a decrease in GL261 cell activity to approximately 50%. © 2026 The Author(s). *Biofuels, Bioproducts and Biorefining* published by Society of Industrial Chemistry and John Wiley & Sons Ltd.

Key words: bioproducts; circular economy; biomass; guaraná seed

Introduction

Guaraná (*Paullinia cupana*) is a fruit native to the Amazon region. The species has potential due to its properties which are important in different industries.[1] Commercially, its main application is in the soft drinks industry, but it is also highly valued in the pharmaceutical and cosmetics industries.[2] The guaraná fruit consists of a red skin with a seed inside, which is partially covered by a white aril. The seed is the only part of the fruit that is consumed and marketable, due to its bioactive content, which includes methylxanthines (caffeine, theobromine, theophylline) and polyphenols.[3]

In 2022, total guaraná production in Brazil stood at 2400 t, with an average productivity of 237 kg ha^{-1}.[4] As the seed is the only part of the fruit of commercial interest, the guaraná production chain generates byproducts such as the husk, peel, and depleted seed. After the guaraná fruit has been harvested, the seeds are pulped to separate them from the peel and aril. The seeds are then roasted and the husk is removed.[5] In the beverage industry, the roasted seed is subjected to hydroalcoholic extraction to obtain the extract, a process that produces the depleted seed as waste.[6]

The byproducts of the guaraná production chain represent biomass with potential for exploitation.[7] Santana and Macedo[8] investigated the performance of hydroalcoholic extraction and aqueous enzymatic extraction for the recovery of catechins and methylxanthines from depleted guaraná seeds and found that the processes were promising. Pinho *et al.*[9] evaluated the extraction of carotenoids from guaraná peel and determined that the antioxidant capacity of the extract was high. Pinho *et al.*[10] evaluated the microencapsulation of guaraná peel extract rich in carotenoids, a process that improved stability during storage. Studies such as these should be carried out more frequently, as they add value to the guaraná production chain, which has underutilized biomass that could have been used to extract different bioproducts.

The appropriate use of byproducts from the guaraná production chain, through the recovery of higher value bioproducts with untapped distribution and marketing potential, could integrate the guaraná production chain into the circular economy, as agricultural waste is considered a sustainable resource for value addition. Pinho *et al.*[9] identified the composition of guaraná peel as 9.7% water, 85.5% carbohydrates, 2.3% proteins, and 1.7% lipids. Despite the predominance of carbohydrates in guaraná peel, studies exploring its minor components could reveal potential uses for this byproduct. Studies into the extraction of vegetable oils and oleoresins have shown the presence of bioactive compounds in these bioproducts, associated with functional properties such as antioxidant and antimicrobial activity.[11,12]

Avato *et al.*[13] evaluated the chemical composition of oil extracted from guaraná seeds. They reported the presence of acylglycerols, cyanolipids, and fatty acids, including oleic, *cis*-11-octadecenoic, and *cis*-11-eicosenoic acids. Benoni *et al.*[14] verified bioactive compounds such as carvacrol, α-copaen, and limonene in guaraná essential oil. These compounds are known for their antioxidant and anti-

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inflammatory properties.[15–20] A study using roasted guaraná seeds also found that guaraná extracts can act as a powerful antioxidant *in vivo*, exhibiting anti-aging properties.[21]

To add value to the guaraná production chain, this study aimed to extract oil from guaraná peel (derived from the removal of the peel and pulp) and guaraná husk (from the removal of the seed husk after roasting), characterize it by gas chromatography–mass spectrometry (GC–MS), and evaluate its biological potential through cytotoxicity and antitumor assays. To the best of our knowledge, this is the first study to identify compounds in guaraná byproducts and evaluate their biological potential.

Material and methods

Sample preparation

The guaraná peel samples were collected manually from fresh guaraná fruit in the municipality of Urucará, Amazonas. The peels were dried in an air-circulating oven (NOVA ÉTICA-400-9D 300 °C, Sao Paulo, Brazil) at 50 °C for 24 h to remove excess moisture. The dried sample was ground in a mill (Lucadema – Luca-226/2, Sao Paulo, Brazil) equipped with a 30 mesh sieve (595 µm aperture) to obtain fine powder. The resulting powder was sieved again through the same mesh to ensure particle size homogeneity and stored in sealed containers until analysis. Compositional and oil extraction analyses were carried out on the homogeneous powdered sample.

The husk samples, manually separated from roasted guaraná seeds collected in the municipality of Urucará, Amazonas, were ground under the same conditions (30 mesh, 595 µm) to obtain a uniform powdered husk fraction used for composition and oil extraction assays.

Composition

Moisture content was determined following the NREL/TP-510-42621 procedure, and ash content was determined following the NREL/TP-510-42622 procedure, as described by Sluiter *et al.*[22] Lipids were measured according to AOCS[23] method Ba 3–38, and protein content was determined by the Kjeldahl method (AOAC 920.87).[24]

Extraction of oils from guaraná peel and husk

The process consisted of solid–liquid extraction using hexane in a Soxhlet apparatus (500 mL). To extract the oils from guaraná peel and husk, the extractor system was refluxed for around 6 h. The extract in the flask was then recovered from the solvent in a rotary evaporator under vacuum (Tecnal-TE-211, Piracicaba, Brazil). The lipids extracted from both samples were stored separately in glass vials wrapped in aluminum foil to protect against light and frozen in a freezer for later analysis.

Gas chromatography–mass spectrometry

The extracted oils were characterized by GC–MS after fatty acid methylation to obtain their respective fatty acid methyl esters, as described by O'Fallon *et al.*[25] In this step, 40 µL of oil was added to a glass tube with 0.7 mL of KOH (10 mol L-1) and 5.3 mL of methanol. After stirring, the mixture was heated at 55 °C for 1.5 h in a shaker (TE-424, Refrigerated Incubator with shake, TECNAL), with the tube shaken every 10 min for 10 s in a vortex. The tube was then removed and cooled to room temperature in cold tap water, followed by the addition of 0.58 mL of H_2SO_4 (12 mol L-1). The tube was heated again (55 °C for 1.5 h), after which 3 mL of hexane was added, vortexed for 5 min, and centrifuged at 2500 *g* for 5 min. The supernatant containing fatty acid methyl esters was then collected and analyzed by GC. The supernatant was analyzed on an Agilent 7890A GC–MS (Agilent Technologies, Santa Clara, CA, USA). The column used was an HP-5MS. The compounds were identified by area percentage.

Cell culture

Sample preparation

The oil samples were resuspended in dimethyl sulfoxide (DMSO), filtered through a 0.22 µm membrane, and diluted in culture medium to obtain different concentrations (0–1000 µg mL^{-1}). All treatments were prepared in quadruplicate.

Cytotoxicity by evaluation of metabolic activity

Cell viability after direct contact with extracts was evaluated with a murine cell line of fibroblasts (L929, Thermo Scientific, Sao Paulo, Brazil) seeded in 96-well cell culture plates (Corning Life Sciences, Durham, USA). Cells were kept under a moist atmosphere at 37 °C with 5% CO_2, and cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cell culture medium was refreshed every 2 days until cells reached 90% to 95% confluence. Cells were detached using Tryplex and placed in 96-well plates at 10 000 cells per well. After 24 h of cell adhesion, different extract concentrations were added to the cell wells. The cells were then incubated at 37 °C, 5% CO_2,

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and 90% humidity for 24, 48, and 72 h. After that, the cells were rinsed with phosphate buffered saline (PBS) (Thermo Fisher Scientific, Sao Paulo, Brazil), and cell viability was measured with the Aqueous One solution proliferation assay (CellTiter 96, MTS, Promega, Madison, USA). In each well, a mixture of 20 µL cell MTS reagent (Promega Corporation, Madison, USA) and 100 µL medium was added and cultured in an incubator (Ultrasafe HF 212UV, Sao Paulo, Brazil) at 37 °C for 2 h with 5% CO_2. The remaining medium was then transferred into 96-well plates for optical density measurements at 490 nm wavelength. The analyses were performed on a spectrophotometer (Molecular Devices, Spectra Max Plus 348, San Jose, USA) with four parallel replicates for each sample. The culture medium was used as a control group, and the results showed metabolic activity.[26]

Antitumor activity

Rat glioma cells (C6), lung adenocarcinoma cells (A549), and mouse glioma cells (GL261) were seeded in 96-well plates at a density of 10 000 cells per well and kept for 24 h at 37 °C in a CO_2 incubator for attachment. After 24 h, the cells were treated with 500 µg mL^{-1} of extracts. Doxorubicin (10 µg mL^{-1}) was used as a positive control, DMSO was the negative control, and the blank control used only the culture medium. The treated cells were incubated for 24 and 48 h at 37 °C in a CO_2 incubator. After incubation, the culture medium was removed, and the cells were washed twice with PBS. The cell viability of extracts was measured using the MTS colorimetric assay as described in the previous section.[27]

Statistical analysis

Metabolic activity of L929 cells was analyzed using OriginPro (OriginLab Corporation, Northampton, MA, USA), and expressed as the mean ± standard error from three independent assays and their triplicates. Statistical evaluation was performed using one-way analysis of variance (ANOVA), followed by Tukey's test with the statistical significance level at $P < 0.05$.

Results and discussion

Proximate composition of guaraná byproducts

Fresh guaraná peel, before drying at 50 °C, had a moisture content of 82.84% ± 0.56%. The lipid content was 1.70% on a dry basis. In a study evaluating the potential of guaraná peel extract as a source of bioactive and as a natural antioxidant, a moisture content of 84.50% was observed, demonstrating the importance of drying to avoid enzymatic activity that can affect the chemical stability of bioactive compounds.[9]

After drying the guaraná peel, the moisture content was 12.39% ± 0.03%, the ash content was 1.30% ± 0.02%, the proteins were 8.04% ± 0.39%, and the lipids were 10.78% ± 0.82%. Morais *et al.*[28] evaluated the peels of several tropical fruits after drying and reported values between 6.4% and 16.9% of proteins and between 0.7% and 5.9% of lipids, respectively. Romelle *et al.*[29] reported lipid values between 3.4% and 12.6% when evaluating the peel of several fruits, closer to those reported in our study.

For the guaraná husk, the seed husk after the roasting process, the moisture values were 7.86% ± 0.10%, the ash values were 3.45% ± 0.15%, and the lipid values were 2.80% ± 0.12%. No data on the characterization of the husk was found in the literature. In a study by Santana and Macedo,[8] the composition obtained with the residual guaraná seed was 10.82% moisture, 1.6% ash, and 6.25% lipids.

Gas chromatography–mass spectrometry

Guaraná peel oil

Figure 1 presents the chromatogram obtained in the GC–MS analysis for the oil extracted from the guaraná peel. Peaks were identified at the initial retention times (20–30 min) related to low molecular weight sesquiterpene compounds. The peaks at the final times (above 60 min) are related to the higher molecular weight compounds. Table 1 identifies the compounds related to the peaks highlighted in the chromatogram, including the peak areas, Chemical Abstracts Service (CAS) number, molecular formula, and molar mass of each compound.

Peak 1 presented a CAS number corresponding to α-copaene, a tricyclic sesquiterpene with broad application prospects in the pharmaceutical, food, and agricultural industries. Studies with essential oils rich in this compound reported antioxidant, antimicrobial, antimutagenic,[30] analgesic, anti-inflammatory,[31] hepatoprotective,[32] and antiplasmodial[33] properties. It is a bioactive phytochemical that occurs naturally in the essential oils of many medicinal and aromatic plants, such as *Piper cubeba*, *Vateria copallifera*, and *Cinnamomum zeylanicum*.[34]

Peak 2 corresponds to caryophyllene, a natural bicyclic sesquiterpene. Among its various biological effects caryophyllene displays anti-inflammatory activity by inhibiting key inflammatory mediators. Many *in vitro* and *in vivo* studies indicate that treatment with caryophyllene improves phenotypes of animals used to model various inflammatory pathologies, such as diseases of the nervous system (Parkinson's disease, Alzheimer's disease, multiple

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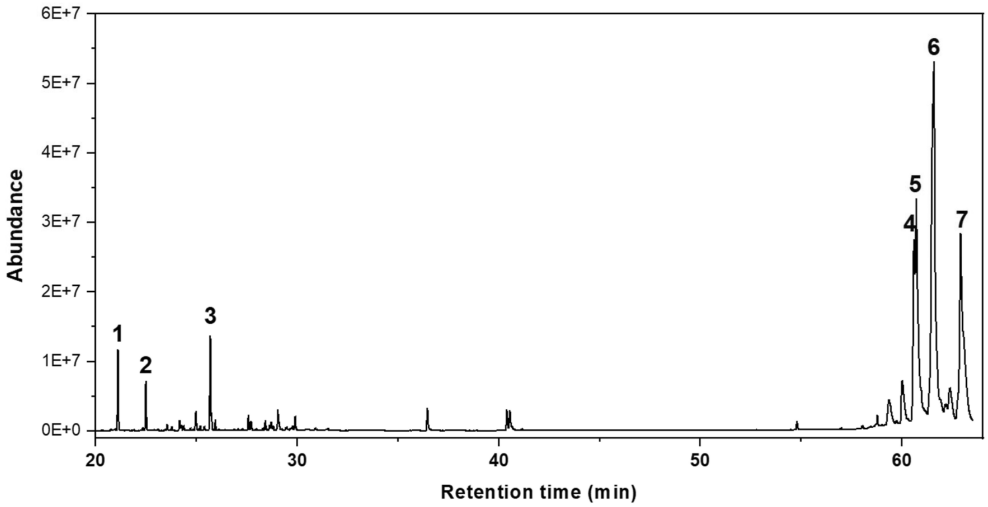


Figure 1. Chromatogram of oil extracted from guaraná peel using gas chromatography–mass spectrometry (GC–MS).

Table 1. Compounds identified in the chromatogram for the guaraná peel using gas chromatography–mass spectrometry (GC–MS).

Peak	Area (%)	Compound	Chemical Abstracts Service (CAS) number	Molecular formula	Molar mass (g mol^{-1})
1	1.7353	α-Copaene	3856-25-5	$C_{15}H_{24}$	204.35
2	1.0328	Caryophyllene	000087-44-5	$C_{15}H_{24}$	204.35
3	2.5032	δ-Cadinene	000483-76-1	$C_{15}H_{24}$	204.35
4	7.5758	9,19-Cyclocholest-24-en-3-ol,14-methyl-, (3 β,5 α)-	068654-82-0	$C_{28}H_{46}O$	398.70
5	16.1428	(E)-14 α-Methyl-5 α-Ergosta-8,23-dien-3 β-ol	090195-44-1	$C_{29}H_{48}O$	412.00
6	33.869	8-Androsten-3-ol, 17-(2-methylallyl)-4,4,14-trimethyl-	1000197-34-3	$C_{28}H_{44}O_2$	412.60
7	17.4761	Cycloartenol	000469-38-5	$C_{30}H_{50}O$	426.70

sclerosis, amyotrophic lateral sclerosis, stroke),[35–38] atherosclerosis and tumors (colon, breast, pancreatic cancer, lymphoma, melanoma, and glioma).[39–45]

Peak 3 corresponds to δ-cadinene, a bicyclic sesquiterpene, usually extracted from the plant species *Eupatorium adenophorum*, native to Mexico.[46,47] Studies have reported that the sesquiterpene cadinene extracted from this plant species has antifungal,[48] antimicrobial,[49] antiviral,[50] antiparasitic,[51] antioxidant,[48] and anticancer[52] effects.

The oleoresin of the copaiba tree, which has been widely studied, is composed of volatile oil formed mainly by sesquiterpene hydrocarbons, such as low molecular weight compounds identified in the chromatogram (Fig. 1): α-copaene, caryophyllene, and δ-cadinene.[53] Most of the bioactive properties attributed to copaiba oleoresin are caused by the action of these compounds. The presence of different sesquiterpenes in the chromatogram of the lipid extracted from guaraná peel demonstrates the potential of this bioproduct, as studies show that sesquiterpenes are bioactive, with a wide range of biological effects, including anti-inflammatory, antimicrobial, antitumor, antimalarial, immunomodulatory properties, and pesticide action.[54]

Peak 4 corresponds to 9,19-cyclocholest-24-en-3-ol, 14-methyl-, (3 β,5 α)-, a compound not found in the literature regarding its presence in raw materials. Peak 5 indicates (E)-14 α-methyl-5 α-ergosta-8,23-dien-3 β-ol. There is little information in the literature regarding its functions. This compound was identified by GC–MS of leaves of the plant species *Telfairia occidentalis*, with a peak area of 2.09% at a retention time of 23.967 min.[55] The most relevant peak in the chromatogram is 6, referring to 8-androsten-3-ol, 17-(2-methylallyl)-4,4,14-trimethyl-, a compound identified in chromatograms of different plant species such as *Terminalia chebula* (peak area 0.81%)[56] and *Allium cepa* L. (peak area 7.78%),[57] *Euphorbia inarticulata* (peak area 3.45%),[58] and others. However, none of the studies cited showed that this compound presented a significant peak, and in-depth discussions about the compound are scarce in the literature.

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Peak 7 refers to cycloartenol, a phytosterol compound and precursor of the biosynthesis of several sterol compounds. It has anti-inflammatory, antitumor, antioxidant, antibiosis, and anti-Alzheimer properties. Cycloartenol also acts in plant growth and development.[59]

Guaraná husk oil

Figure 2 presents the chromatogram obtained in the GC–MS analysis for the oil extracted from the guaraná husk. Peaks were identified at retention times of 30–40 min, corresponding to low-molecular-weight compounds (Table 2).

Table 2 identifies the compounds related to the peaks highlighted in the chromatogram.

Peak 1, one of the major peaks, was identified as palmitic acid methyl ester (methyl palmitate), a fatty acid methyl ester derived from lipids. This compound is reported as a major antimicrobial and antifungal agent in microalgal fatty acids[60,61] in *Celtis australis* L. fruit extract,[62] *Andrographis paniculata* root oil,[63] *Halimodendron halodendron* flower oil,[64] and oils from three *Phlomis* species.[65] Palmitic acid methyl ester has also been associated with anticancer activity,[66] neuroprotection against cardiac arrest,[67,68] prevention of cardiac hypertrophy,[69] and protection against nonalcoholic steatohepatitis.[70] These properties indicate that guaraná husk oil may have considerable potential for therapeutic applications.

Peak 2, found in guaraná husk oil – linoleic acid methyl ester (or methyl linoleate) – was verified in several matrices, such as soybean, corn, and sunflower vegetable oil,[71,72] flixweed (*Descurainia sophia* L.) seed,[73] *Anacyclus cyrtolepidioïdes* (Pomel),[74] quince (*Cydonia oblonga* Miller) seed,[75] and macroalgae (*Gracilariopsis persica*).[76] It is known for its antibacterial and antifungal effects.[71,76,77]

Peak 3 was identified as 7-octadecenoic acid methyl ester, primarily known for its antibacterial activity, and has been reported in several matrices, including *Guiera senegalensis* root,[78] Sudanese *Ziziphus spina-christi* seeds,[79] *Cassytha filiformis* L. (Lauraceae),[80] *Trachyspermum ammi* seeds,[81] and leaves of *Psidium guajava* Linn.[82] Peak 4, 11-octadecenoic acid methyl ester, has been found in Nigerian *Vigna radiata* root,[83] *Acacia nilotica* Linn seeds,[84] *Celtis integrifolia* Lam,[85]

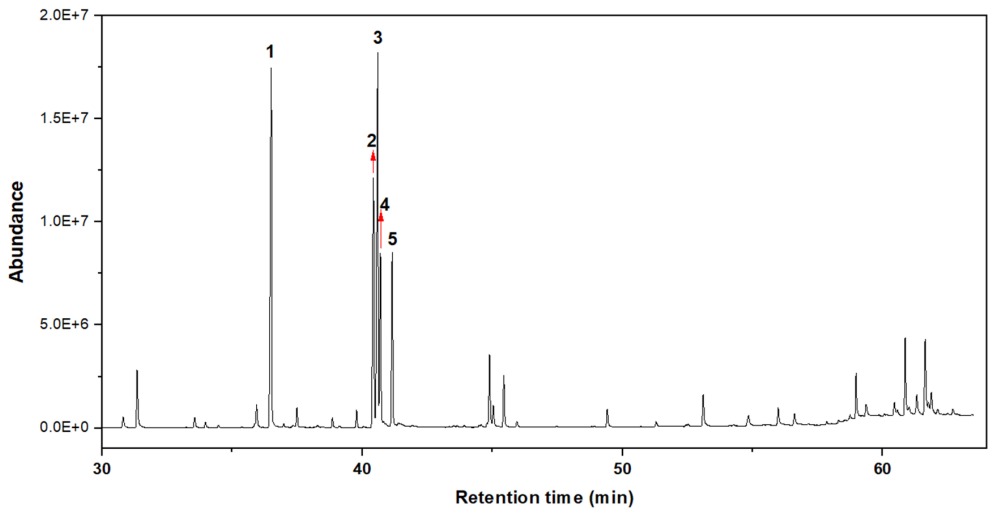


Figure 2. Chromatogram of oil extracted from guaraná husk using gas chromatography–mass spectrometry (GC–MS).

Table 2. Compounds identified in the chromatogram for guaraná husk using gas chromatography–mass spectrometry (GC–MS).

Peak	Area (%)	Compound	Chemical Abstracts Service (CAS) number	Molecular formula	Molar mass (g mol^{-1})
1	20.3857	Palmitic acid, methyl ester	000112-39-0	$C_{17}H_{34}O_2$	270.54
2	13.4011	Linoleic acid, methyl ester	000112-63-0	$C_{19}H_{34}O_2$	294.74
3	22.578	7-Octadecenoic acid, methyl ester	057396-98-2	$C_{19}H_{36}O_2$	296.48
4	8.1153	11-Octadecenoic acid, methyl ester	052380-33-3	$C_{19}H_{36}O_2$	296.48
5	8.8406	Stearic acid, methyl ester	000112-61-8	$C_{19}H_{38}O_2$	298.5

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and the stem bark of *Mangifera indica* and *Azadirachta indica*.[86] According to Kwaji and Martin,[85] this methyl fatty acid exhibits broad-spectrum antibacterial activity, particularly against *Escherichia coli*, and *Shigella dysenteriae*.

The last compound verified by the chromatogram for guaraná husk oil is stearic acid methyl ester (peak 5), one of the most common fatty acids (along with palmitic). It has been found in several matrices, such as mango (*Mangifera indica* L.) kernel oil,[87] marine fish oils from Indonesia,[88] *Abutilon hirtum* (Lam.) sweet leaves[89] and walnut oils.[90] This acid has been reported to accelerate cartilage defect repair, provide neuroprotection, improve functional outcomes after cardiac arrest,[91] and serve as a surface modifier in polymer formulations and composite processing.[92]

In general, both oils contain fatty acids with strong potential, which has already been demonstrated. However, cytotoxicity analyses are necessary to ensure that they are not harmful to human health. Possible cytoprotective and antitumor potential must also be evaluated.

Cytotoxicity effects of guaraná oil byproducts

Cytotoxicity assessment is essential for a wound-healing product, as it helps understand contact with infected wound tissue and its neighboring eukaryotic cells.[93] As Fig. 3 shows, the cytotoxicity of guaraná peel and husk oils was assessed in L929 cells. Guaraná peel oil at 250 $\mu g\,mL^{-1}$ maintained 73.8% metabolic activity, whereas guaraná husk oil at 100 $\mu g\,mL^{-1}$ maintained 105%. At concentrations above these levels (> 250 $\mu g\,mL^{-1}$), the oils exhibit cytotoxicity in L929 cells, according to ISO 10993-5:2009[94]; in which the minimum cell viability is 70%.

These results are important to ensure the use of oil concentrations that are not cytotoxic and to evaluate other potential effects, such as antitumor activity.

In the study by Marinas *et al*.,[95] essential oil extracted from *Amorpha fruticosa* fruits showed just over 80% L929 cell viability at 288 $\mu g\,mL^{-1}$, but viability dropped to approximately 10% at 576 $\mu g\,mL^{-1}$. Javanshir *et al*.[96] observed cytotoxicity (viability <70%) at 62.5 $\mu g\,mL^{-1}$ of *Ricinus communis* L. essential oil nanoemulsions. Different oils therefore exhibit varying cytotoxicity. In our study, guaraná peel and husk oils also differed in cytotoxicity, primarily due to their distinct lipid profiles, as shown in Tables 1 and 2.

Antitumor effects of guaraná byproduct oils

An antitumor (or antiproliferative) effect was observed for both oils (Fig. 4). As the concentrations of both oils increased, the metabolic activity of all tested cells decreased. For C6 cells, at concentrations that did not induce cytotoxicity (250 $\mu g\,mL^{-1}$ for peel oil and 100 $\mu g\,mL^{-1}$ for husk oil), both oils reduced metabolic activity to 66.9% and 21.3%, respectively. In A459 cells, metabolic activity at the same concentrations decreased to 65.2% for peel oil and 75.8% for husk oil. For GL261 cells, metabolic activity was 52.8% for peel oil and 48.0% for husk oil. Both oils demonstrated potential antitumor effects, with husk oil showing the greatest impact in C6 cells, peel oil in A459 cells, and both oils in GL261 cells.

As mentioned above, some of the major compounds present in the peel oil (peaks 5 and 6 in Fig. 1) have not yet been studied, so there is still little information about their antitumor properties. However, cycloartenol, another major compound, showed an antiproliferative effect on glioma U87 cells, indicating an anticancer effect.[97] Zaghlol *et al*.[98] found that cycloartenol was present in *Euphorbia greenwayi* extracts. These fractions showed antitumor activity for MCF-7, HepG-2, and SW620 cells.

Among the main compounds present in guaraná husk oil (Table 2), 7-octadecenoic acid methyl ester (22.6%) and palmitic acid methyl ester (20.4%) were the majority.

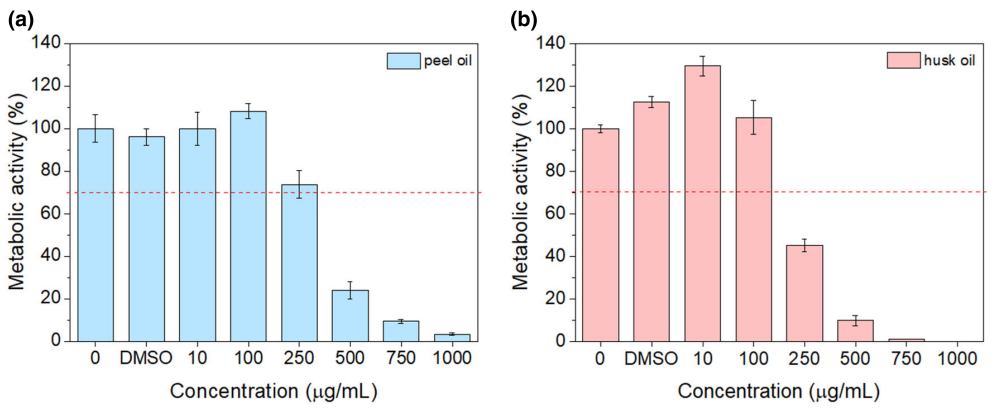


Figure 3. Metabolic activity of L929 cells treated for 24 h with (a) guaraná peel oil and (b) guaraná husk oil.

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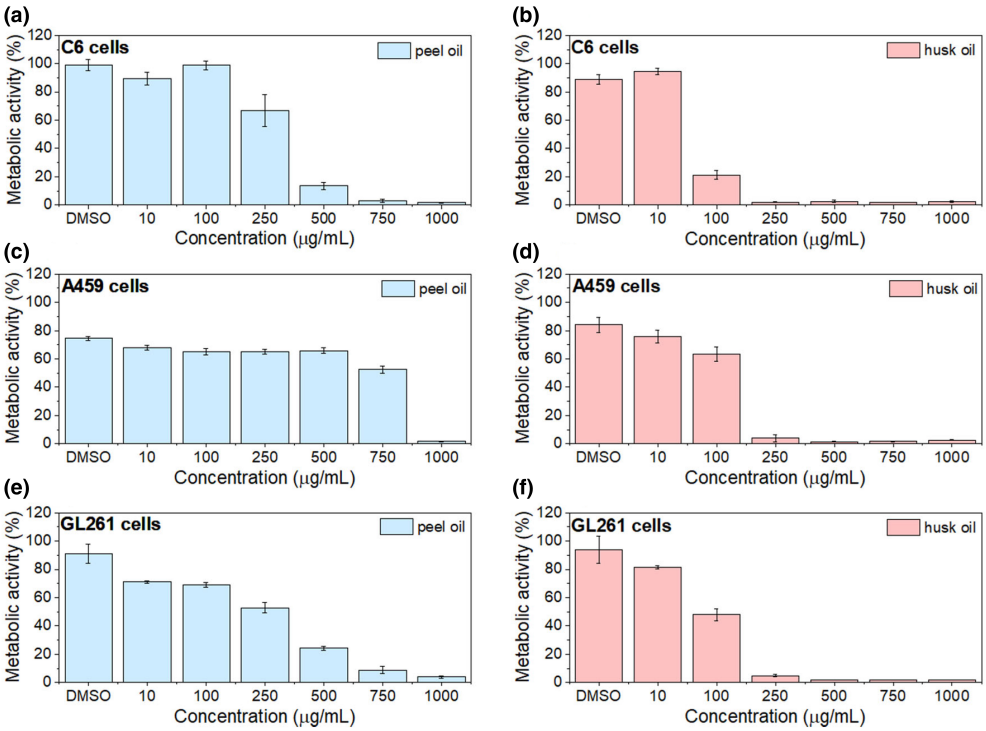


Figure 4. Antitumor activity (%) of guaraná peel oil (a, c, e) and guaraná husk oil (b, d, f) in rat glioma cells (C6), lung adenocarcinoma cells (A549), and mouse glioma cells (GL261).

Leyva-Peralta *et al.*[99] reported that an active fraction obtained with ethyl acetate (FAg-4A) from *Argemone gracilenta* exhibited antitumor activity against M12.C3F6, RAW 264.7, HeLa, and L929 cells, with 7-octadecenoic acid methyl ester as the major compound (40.8%). Mota *et al.*[100] also identified 7-octadecenoic acid methyl ester (10.4%) and palmitic acid methyl ester (4.9%) in extracts of *Passiflora edulis* f. *flavicarpa* Deg. They proposed that the antitumor activity of medium- and long-chain fatty acids may result from mitochondrial damage leading to intracellular overproduction of reactive oxygen species (ROS), which causes oxidative damage to lipids, proteins, and DNA, cell cycle arrest, and apoptosis.

Zafaryab *et al.*[101] verified the antitumor effect of palmitic acid on MCF-7 breast cancer cells. Lin *et al.*[66] found that palmitic acid methyl ester could increase *p53* expression in the A549 cancer cell line (inducing both cell cycle arrest and cell death) and reduce human bone marrow-derived MSCs, a potential application in treating cancerous diseases. Djeghim *et al.*[102] reported that oleic acid, palmitic acid, and stearic acid exhibited antiproliferative activity against several cancer cell lines, but further research is needed to analyze pathways and gene expression profiles to fully elucidate their mechanisms of action and ultimately establish their true therapeutic potential.

Although the biological activity of guaraná oil has not been reported previously, the synergistic effect of its main fatty acids may exert an antitumor effect on the tested cells. Future studies should be conducted to elucidate these effects and to investigate the potential of these oils as alternative anticancer agents or for treatment in patients with glioma.

Conclusion

Reusing guaraná byproduct strengthens its production chain and supports the circular economy. Oil was obtained from both the peel and the husk during guaraná processing. The oils had distinct compositions: the main compound in peel oil was 8-androsten-3-ol, 17-(2-methylallyl)-4,4,14-trimethyl, whereas the major compounds in husk oil were 7-octadecenoic acid methyl ester and palmitic acid methyl ester. Cytotoxicity analysis in L929 cells showed that concentrations of 250 and 100 $\mu g\,mL^{-1}$ reduced metabolic activity by more than 70%, indicating cytotoxicity. At noncytotoxic concentrations, both oils exhibited antitumor activity: husk oil was most effective in C6 cells, peel oil in A459 cells, and both oils showed similar effects in GL261 cells.

Overall, oils from guaraná peel and husk exhibited *in vitro* antitumor effects and may offer a strategy for valorizing waste in the food and pharmaceutical industries. However, the

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molecular mechanisms underlying their antitumor activity remain unclear, including whether they induce apoptosis, inhibit proliferation, arrest the cell cycle, or affect cell migration and invasion.

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References

1. Salles RC d O, Muniz MP, Nunomura R d CS and Nunomura SM, Geographical origin of guarana seeds from untargeted UHPLC-MS and chemometrics analysis. *Food Chem* **371**:131068 (2022). https://doi.org/10.1016/j.foodchem.2021.131068.
2. Marques LLM, Ferreira EDF, de Paula MN, Klein T and de Mello JCP, *Paullinia cupana*: A multipurpose plant – A review. *Rev Bras Farmacogn* **29**(1):77–110 (2019). https://doi.org/10.1016/j.bjp.2018.08.007.
3. Santana ÁL and Macedo GA, Health and technological aspects of methylxanthines and polyphenols from guarana: A review. *J Funct Foods* **47**:457–468 (2018). https://doi.org/10.1016/j.jff.2018.05.048.
4. Conab, Histórico Mensal do Guaraná. (2023). Available: https://www.conab.gov.br/info-agro/analises-do-mercado-agropecuario-e-extrativista/analises-do-mercado/historico-mensal-de-Guarana.
5. SUFRAMA, *Projeto Potencialidades Regionais Estudo de Viabilidade Econômica*. Superintendency of the Manaus Free Trade Zone (SUFRAMA) in partnership with the Getúlio Vargas Foundation (FGV) and the Higher Institute of Economics and Administration (ISAE), Manaus, Brazil (2003). Available: http://www.suframa.gov.br/publicacoes/proj_pot_regionais/guarana.pdf.
6. Santana ÁL, Zanini JA and Macedo GA, Dispersion-assisted extraction of guarana processing wastes on the obtaining of polyphenols and alkaloids. *J Food Process Eng* **43**(4): 13381–13392 (2020). https://doi.org/10.1111/jfpe.13381.
7. de Teixeira Azevedo G, Lima Souza G, Leonarski E, Lotas KM, da Barroso Silva GH, Miguel Batista FR *et al*., Valorization of guaraná (*Paullinia cupana*) production chain waste – A review of possible bioproducts. *Resources* **14**(6):98 (2025). https://doi.org/10.3390/resources14060098.
8. Santana ÁL and Macedo GA, Effects of hydroalcoholic and enzyme-assisted extraction processes on the recovery of catechins and methylxanthines from crude and waste seeds of guarana (*Paullinia cupana*). *Food Chem* **281**:222–230 (2019). https://doi.org/10.1016/j.foodchem.2018.12.091.
9. Pinho LS, Silva MP, Thomazini M, Cooperstone JL, Campanella OH, Costa Rodrigues CE *et al*., Guaraná (*Paullinia cupana*) by-product as a source of bioactive compounds and as a natural antioxidant for food applications. *J Food Process Preserv* **45**(10) :15854–15863 (2021). https://doi.org/10.1111/jfpp.15854.
10. Pinho LS, Patel BK, Campanella OH, Rodrigues CE d C and Favaro-Trindade CS, Microencapsulation of carotenoid-rich extract from Guaraná peels and study of microparticle functionality through incorporation into an oatmeal paste. *Foods* **12**(6) :1170–1185 (2023). https://doi.org/10.3390/foods12061170.
11. Gopika SK and Subramanian R, Recovery of bioactive volatiles from byproduct of curcumin manufacture by membrane processing. *Ind Crops Prod* **158**:112984 (2020). https://doi.org/10.1016/j.indcrop.2020.112984.
12. Song Y, Zhang W, Wu J, Admassu H, Liu J, Zhao W *et al*., Ethanol-assisted aqueous enzymatic extraction of Peony seed oil. *J Am Oil Chem Soc* **96**(5):595–606 (2019). https://doi.org/10.1002/aocs.12204.
13. Avato P, Pesante MA, Fanizzi FP and Santos CA d M, Seed oil composition of *Paullinia cupana var. sorbilis* (Mart.) Ducke. *Lipids* **38**(7):773–780 (2003). https://doi.org/10.1007/s11745-003-1126-5.
14. Benoni H, Lamaty G, Menut C, Bessière JM and Sauvan N, Studies on the essential oil from guaraná (*Paullinia cupana* var. *sorbilis*). *Z Lebensm Unters Forsch* **203**(1):95–98 (1996). https://doi.org/10.1007/BF01234330.
15. Pangi VN, Marukurti A, Reddy AM, Anupoju R, Kasi PB, Medapalli SR *et al*., Anti-vibriocidal activity and gas chromatography mass spectrometry (GC-MS)-based chemical composition of *Mirabilis jalapa* leaf methanolic extract. *J Pharmacogn Phytochem* **12**(1):653–662 (2023). Available: https://www.phytojournal.com.
16. Santa-María C, López-Enríquez S, de la Montserrat- Paz S, Geniz I, Reyes-Quiroz ME, Moreno M *et al*., Update on anti-inflammatory molecular mechanisms induced by oleic acid. *Nutrients* **15**(1):224 (2023). https://doi.org/10.3390/nu15010224.
17. Thiagalingam V, Chandrasekar S, Boopathy U and Durairaj R, Evaluation of bioactive compounds in *Desmidorchis indica* stem extract using spectroscopic and chromatographic techniques. *Int J Membr Sci Technol* **10**(2):2246–2259 (2023). https://doi.org/10.15379/ijmst.v10i2.2809.
18. Carvalho FO, Silva ÉR, Gomes IA, Santana HSR, Santos DN, Souza GPO *et al*., Anti-inflammatory and antioxidant activity of carvacrol in the respiratory system: A systematic review and meta-analysis. *Phytother Res* **34**(9):2214–2229 (2020). https://doi.org/10.1002/ptr.6688.
19. Scur MC, Pinto FGS, Pandini JA, Costa WF, Leite CW and Temponi LG, Antimicrobial and antioxidant activity of essential oil and different plant extracts of *Psidium cattleianum* Sabine. *Braz J Biol* **76**(1):101–108 (2016). https://doi.org/10.1590/1519-6984.13714.
20. Santana HSR, Carvalho FO, Silva ÉR, Santos NGL, Shanmugam S, Santos DN *et al*., Anti-inflammatory activity of limonene in the prevention and control of injuries in the respiratory system: A systematic review. *Curr Pharm Des* **26**(18):2182–2191 (2020). https://doi.org/10.2174/1381612826666200320130443.
21. Peixoto H, Roxo M, Röhrig T, Richling E, Wang X and Wink M, Anti-aging and antioxidant potential of *Paullinia cupana var. sorbilis*: Findings in *Caenorhabditis elegans* indicate a new utilization for roasted seeds of guaraná. *Medicine* **4**(3):61 (2017). https://doi.org/10.3390/medicines4030061.

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22. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D *et al*., *Determination of Structural Carbohydrates and Lignin in Biomass – NREL/TP-510-42618*. National Renewable Energy Laboratory, Golden (2008). Available: http://www.nrel.gov/docs/gen/fy13/42618.pdf.
23. American Oil Chemist's Society, *Official Methods and Recommended Practices of the AOCS*, 6th edn. AOCS, Champaign (2009).
24. AOAC International, *Official Methods of Analysis of AOAC International*. Association of Official Analysis Chemists International, Champaign, p. 3172 (2016).
25. O'fallon JV, Busboom JR, Nelson ML and Gaskins CT, A direct method for fatty acid methyl ester synthesis: Application to wet meat tissues, oils and feedstuffs. *J Anim Sci* **85**:1511–1521 (2007).
26. Aguilar AEM, Fagundes AP, Macuvele DLP, Cesca K, Porto L, Padoin N *et al*., Green synthesis of Nano hydroxyapatite: Morphology variation and its effect on cytotoxicity against fibroblast. *Mater Lett* **284**:129013 (2021). https://doi.org/10.1016/j.matlet.2020.129013.
27. Sousa MHO, Morgan JMS, Cesca K, Flach A and de Moura NF, Cytotoxic activity of *Cunila angustifolia* essential oil. *Chem Biodivers* **17**(2):3–7 (2020). https://doi.org/10.1002/cbdv.201900656.
28. Morais DR, Rotta EM, Sargi SC, Bonafe EG, Suzuki RM, Souza NE *et al*., Proximate composition, mineral contents and fatty acid composition of the different parts and dried peels of tropical fruits cultivated in Brazil. *J Braz Chem Soc* **28**(2):308–318 (2017). https://doi.org/10.5935/0103-5053.20160178.
29. Romelle FD, Rani AP and Sai Manohar R, Chemical composition of some selected fruit peels. *Eur J Food Sci Technol* **4**(4):12–21 (2016). Available: https://www.researchgate.net/publication/326579276.
30. Zahin M, Bokhari NA, Ahmad I, Husain FM, Althubiani AS, Alruways MW *et al*., Antioxidant, antibacterial, and antimutagenic activity of *Piper nigrum* seeds extracts. *Saudi J Biol Sci* **28**(9):5094–5105 (2021). https://doi.org/10.1016/j.sjbs.2021.05.030.
31. Chavan MJ, Wakte PS and Shinde DB, Analgesic and anti-inflammatory activities of the sesquiterpene fraction from *Annona reticulata* L. bark. *Nat Prod Res* **26**(16):1515–1518 (2012). https://doi.org/10.1080/14786419.2011.564583.
32. Vinholes J, Rudnitskaya A, Gonçalves P, Martel F, Coimbra MA and Rocha SM, Hepatoprotection of sesquiterpenoids: A quantitative structure-activity relationship (QSAR) approach. *Food Chem* **146**:78–84 (2014). https://doi.org/10.1016/j.foodchem.2013.09.039.
33. Boyom FF, Ngouana V, Zollo PHA, Menut C, Bessiere JM, Gut J *et al*., Composition and anti-plasmodial activities of essential oils from some Cameroonian medicinal plants. *Phytochemistry* **64**(7):1269–1275 (2003). https://doi.org/10.1016/j.phytochem.2003.08.004.
34. Zhang S, Zhao X, He X, Yang L, Wang Y, Liu F *et al*., Metabolic engineering of *Escherichia coli* for the biosynthesis of α-copaene from glucose. *Biochem Eng J* **186**:1–6 (2022). https://doi.org/10.1016/j.bej.2022.108561.
35. Alberti TB, Barbosa WLR, Vieira JLF, Raposo NRB and Dutra RC, (–)-β-caryophyllene, a CB2 receptor-selective phytocannabinoid, suppresses motor paralysis and neuroinflammation in a murine model of multiple sclerosis. *Int J Mol Sci* **18**(4):1–14 (2017). https://doi.org/10.3390/ijms18040691.
36. Cheng Y, Dong Z and Liu S, β-Caryophyllene ameliorates the Alzheimer-like phenotype in APP/PS1 mice through CB2 receptor activation and the PPARγ pathway. *Pharmacology* **94**(1–2):1–12 (2014). https://doi.org/10.1159/000362689.
37. Ullah H, Di Minno A, Santarcangelo C, Khan H and Daglia M, Improvement of oxidative stress and mitochondrial dysfunction by β-caryophyllene: A focus on the nervous system. *Antioxidants* **10**(4):546–567 (2021). https://doi.org/10.3390/antiox10040546.
38. Viveros-Paredes JM, González-Castañeda RE, Gertsch J, Chaparro-Huerta V, López-Roa RI, Vázquez-Valls E *et al*., Neuroprotective effects of β-caryophyllene against dopaminergic neuron injury in a murine model of Parkinson's disease induced by MPTP. *Pharmaceuticals* **10**(3):60–77 (2017). https://doi.org/10.3390/ph10030060.
39. Assis LC, Straliotto MR, Engel D, Hort MA, Dutra RC and de Bem AF, β-Caryophyllene protects the C6 glioma cells against glutamate-induced excitotoxicity through the Nrf2 pathway. *Neuroscience* **279**:220–231 (2014). https://doi.org/10.1016/j.neuroscience.2014.08.043.
40. Baldissera MD, Souza CF, Grando TH, Stefani LM and Monteiro SG, β-Caryophyllene reduces atherogenic index and coronary risk index in hypercholesterolemic rats: The involvement of cardiac oxidative damage. *Chem Biol Interact* **270**:9–14 (2017). https://doi.org/10.1016/j.cbi.2017.04.008.
41. Cho JY, Kim HY, Kim SK, Park JHY, Lee HJ and Chun HS, β-Caryophyllene attenuates dextran sulfate sodium-induced colitis in mice via modulation of gene expression associated mainly with colon inflammation. *Toxicol Rep* **2**:1039–1045 (2015). https://doi.org/10.1016/j.toxrep.2015.07.018.
42. Francomano F, Caruso A, Barbarossa A, Fazio A, La Torre C, Llamaci R *et al*., β-Caryophyllene a sesquiterpene with countless. *Appl Sci* **9**:5420–5438 (2019).
43. Hanušová V, Caltová K, Svobodová H, Ambrož M, Skarka A, Murínová N *et al*., The effects of β-caryophyllene oxide and trans-nerolidol on the efficacy of doxorubicin in breast cancer cells and breast tumor-bearing mice. *Biomed Pharmacother* **95**:828–836 (2017). https://doi.org/10.1016/j.biopha.2017.09.008.
44. Sain S, Naoghare PK, Saravana Devi S, Daiwile A, Krishnamurthi K, Arrigo P *et al*., Beta caryophyllene and caryophyllene oxide, isolated from *Aegle marmelos*, as the potent anti-inflammatory agents against lymphoma and Ne. **13**(1):45–55 (2014).
45. Yang CH, Huang YC, Tsai ML, Cheng CY, Liu LL, Yen YW *et al*., Inhibition of melanogenesis by β-caryophyllene from lime mint essential oil in mouse B16 melanoma cells. *Int J Cosmet Sci* **37**(5):550–554 (2015). https://doi.org/10.1111/ics.12224.
46. Cincotta F, Verzera A, Tripodi G and Condurso C, Determination of sesquiterpenes in wines by HS-SPME coupled with GC-MS. *Chromatography* **2**(3):410–421 (2015). https://doi.org/10.3390/chromatography2030410.
47. Giri S, Sahu R, Paul P, Nandi G and Dua TK, An updated review on Eupatorium adenophorum Spreng. [*Ageratina adenophora* (Spreng.)]: Traditional uses, phytochemistry, pharmacological activities and toxicity. *Pharmacol Res Mod Chinese Med* **2**:100068 (2022). https://doi.org/10.1016/j.prmcm.2022.100068.
48. Kundu A, Saha S, Walia S, Ahluwalia V and Kaur C, Antioxidant potential of essential oil and cadinene sesquiterpenes of *Eupatorium adenophorum*. *Toxicol Environ Chem* **95**(1):127–137 (2013). https://doi.org/10.1080/02772248.2012.759577.
49. Ahluwalia V, Sisodia R, Walia S, Sati OP, Kumar J and Kundu A, Chemical analysis of essential oils of *Eupatorium*

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adenophorum and their antimicrobial, antioxidant and phytotoxic properties. *Journal of Pest Science* **87**(2):341–349 (2014). https://doi.org/10.1007/s10340-013-0542-6.
50. Xu R, Weng J, Hu L, Peng G, Ren Z, Deng J *et al*., Anti-NDV activity of 9-oxo10,11-dehydroageraphorone extracted from *Eupatorium adenophorum* Spreng in vitro. *Nat Prod Res* **32**(18):2244–2247 (2018). https://doi.org/10.1080/14786419.2017.1371158.
51. Nong X, Li SH, Wang JH, Xie Y, Chen FZ, Liu TF *et al*., Acaricidal activity of petroleum ether extracts from *Eupatorium adenophorum* against the ectoparasitic cattle mite, Chorioptes texanus. *Parasitol Res* **113**(3):1201–1207 (2014). https://doi.org/10.1007/s00436-014-3758-3.
52. Liao F, Hu Y, Wu L, Tan H, Luo B, He Y *et al*., Induction and mechanism of HeLa cell apoptosis by 9-oxo-10, 11-dehydroageraphorone from *Eupatorium adenophorum*. *Oncol Rep* **33**(4):1823–1827 (2015). https://doi.org/10.3892/or.2015.3778.
53. da Trindade R, da Silva JK and Setzer WN, Copaifera of the neotropics: A review of the phytochemistry and pharmacology. *Int J Mol Sci* **19**(5):1511–1543 (2018). https://doi.org/10.3390/ijms19051511.
54. Gong DY, Chen XY, Guo SX, Wang BC and Li B, Recent advances and new insights in biosynthesis of dendrobine and sesquiterpenes. *Appl Microbiol Biotechnol* **105**(18):6597–6606 (2021). https://doi.org/10.1007/s00253-021-11534-1.
55. Christopher DT, Willie IE, Akpan IO and Domingo ET, Antioxidant activity and identification of bioactive compounds in *Telfairia occidentalis* leaves using GC-MS analysis. *World J Adv Res Rev* **16**(3):775–788 (2022). https://doi.org/10.30574/wjarr.2022.16.3.1291.
56. Sagar Rajendra C and Prashant Warke S, Formulation and evaluation of herbal nanoemulgel of *Terminalia chebula*. *Int J Creat Res Thoughts* **10**(9):b831–b846 (2022).
57. Galingging RY, Sobir K, Aisyah SI and Maharijaya A, GC-MS profiling of volatile compounds from fifteen different varieties of Indonesian shallot grown in tidal swampland. *Rasayan J Chem* **11**(2):575–581 (2018). https://doi.org/10.31788/RJC.2018.1123001.
58. Almalki G, Rabah S, Al-Faifi Z, Alharbi A and Sharma M, Phytochemistry screening, antioxidant and antimicrobial activities of *Euphorbia inarticulata* Schweinf plant extract. *Pharmacophore* **13**(1):91–99 (2022). https://doi.org/10.51847/s7eqwfebax.
59. Zhang ZL, Luo ZL, Shi H, Zhang L and Ma X, Research advance of functional plant pharmaceutical cycloartenol about pharmacological and physiological activity. *China J Chin Mater Med* **42**(3):433–437 (2017).
60. Davoodbasha MA, Edachery B, Nooruddin T, Lee SY and Kim JW, An evidence of C16 fatty acid methyl esters extracted from microalga for effective antimicrobial and antioxidant property. *Microb Pathog* **115**:233–238 (2018). https://doi.org/10.1016/j.micpath.2017.12.049.
61. Davoodbasha M, Ramasamy P, Thiyagarajan S, Thayalan MN, Abdulazees PA, Naif Abdullah A-D *et al*., New reports on anti-bacterial and anti-candidal activities of fatty acid methyl esters (FAME) obtained from *Scenedesmus bijugatus* var. bicellularis biomass. *RSC Adv* **2**(30):11552 (2012). https://doi.org/10.1039/c2ra21130k.
62. Badoni R, Semwal DK and Rawat U, Fatty acid composition and antimicrobial activity of *Celtis australis* L. fruits. *J Sci Res* **2**(2):397–402 (2010). https://doi.org/10.3329/jsr.v2i2.4056.
63. Farhana S, Aziz S, Rahman S, Afrin S, Bhuiyan MNI and Al-Reza SM, Chemical composition of fixed oil and in vitro antimicrobial activity of *Andrographis paniculata* root. *J King Saud Univ Sci* **34**(4):101921 (2022). https://doi.org/10.1016/j.jksus.2022.101921.
64. Wang J, Liu H, Gao H, Zhao J, Zhou L, Han J *et al*., Antimicrobial and antioxidant activities of the flower essential oil of *Halimodendron halodendron*. *Nat Prod Commun* **6**(11):1749–1753 (2011). https://doi.org/10.1177/1934578x1100601144.
65. Mamadalieva NZ, Youssef FS, Ashour ML, Akramov DK, Sasmakov SA, Ramazonov NS *et al*., A comparative study on chemical composition and antimicrobial activity of essential oils from three *Phlomis* species from Uzbekistan. *Nat Prod Res* **35**(4):696–701 (2021). https://doi.org/10.1080/14786419.2019.1591400.
66. Lin JH, Ting PC, Lee WS, Chiu HW, Chien CA, Liu CH *et al*., Palmitic acid methyl Ester induces G2/M arrest in human bone marrow-derived mesenchymal stem cells via the p53/p21 pathway. *Stem Cells Int* **2019**:1–15 (2019). https://doi.org/10.1155/2019/7606238.
67. Lee RHC, Couto e Silva A, Possoit HLE, Lerner FM, Chen PY, Azizbayeva R *et al*., Palmitic acid methyl ester is a novel neuroprotective agent against cardiac arrest. *Prostaglandins Leukot Essent Fatty Acids* **147**:6–14 (2019). https://doi.org/10.1016/j.plefa.2018.11.011.
68. Wu CYC, Couto e Silva A, Citadin CT, Clemons GA, Acosta CH, Knox BA *et al*., Palmitic acid methyl ester inhibits cardiac arrest-induced neuroinflammation and mitochondrial dysfunction. *Prostaglandins Leukot Essent Fatty Acids* **165**:102227 (2021). https://doi.org/10.1016/j.plefa.2020.102227.
69. Lien CF, Chiu HW, Lee WS, Lin JH, Wang YS, Ting PC *et al*., Palmitic acid methyl ester induces cardiac hypertrophy through activating the GPR receptor-mediated changes of intracellular calcium concentrations and mitochondrial functions. *J Cell Physiol* **238**(1):242–256 (2023). https://doi.org/10.1002/jcp.30922.
70. Zhang L, Li HX, Pan WS, Ullah Khan F, Qian C, Qi-Li FR *et al*., Administration of methyl palmitate prevents non-alcoholic steatohepatitis (NASH) by induction of PPAR-α. *Biomed Pharmacother* **111**:99–108 (2019). https://doi.org/10.1016/j.biopha.2018.12.059.
71. Pinto MEA, Araújo SG, Morais MI, Sá NP, Lima CM, Rosa CA *et al*., Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils. *An Acad Bras Cienc* **89**(3):1671–1681 (2017). https://doi.org/10.1590/0001-3765201720160908.
72. Yong Q, Yuan G and Li H, Extraction and separation of unsaturated fatty acids from sunflower oil. *IOP Conf Ser Earth Environ Sci* **680**(1):012063 (2021). https://doi.org/10.1088/1755-1315/680/1/012063.
73. Ara KM, Jowkarderis M and Raofie F, Optimization of supercritical fluid extraction of essential oils and fatty acids from flixweed (*Descurainia sophia* L.) seed using response surface methodology and central composite design. *J Food Sci Technol* **52**(7):4450–4458 (2015). https://doi.org/10.1007/s13197-014-1353-3.
74. Hammami S, Bergaoui A, Bouzidi A, Ciavatta ML, Cimino G, Abreu PM *et al*., Isolation and structure elucidation of flavonol glycosides, methyl linoleate and fatty acids from *Anacyclus cyrtolepidioïdes* (Pomel) growing in Tunisia. *Anal Chem Lett* **1**(5–6):384–392 (2011). https://doi.org/10.1080/22297928.2011.10648242.
75. Daneshvand B, Ara KM and Raofie F, Comparison of supercritical fluid extraction and ultrasound-assisted extraction of fatty acids from quince (*Cydonia oblonga*

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miller) seed using response surface methodology and central composite design. *J Chromatogr A* **1252**:1–7 (2012). https://doi.org/10.1016/j.chroma.2012.06.063.
76. Pourakbar L, Moghaddama SS, El Enshasy HA and Sayyed RZ, Antifungal activity of the extract of a macroalgae, *Gracilariopsis persica*, against four plant pathogenic fungi in vitro. *Plants* **10**(9):1–13 (2021). https://doi.org/10.3390/plants10091781.
77. Mujeeb F, Bajpai P and Pathak N, Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. *Biomed Res Int* **2014**:1–11 (2014). https://doi.org/10.1155/2014/497606.
78. Shettima AY, Karumi Y, Sodipo OA, Usman H and Tijjani MA, Gas chromatography-mass spectrometry (GC-MS) analysis of bioactive components of ethyl acetate root extract of *Guiera senegalensis* J.F. Gmel. *J Appl Pharm Sci* **3**(3):146–150 (2013). https://doi.org/10.7324/JAPS.2013.30328.
79. Abubaker MA, Mohammed AAA, Farah AAM and Zhang J, Phytochemical screening by using GC-MS and FTIR spectrum analysis of fixed oil from Sudanese *Ziziphus spina Christi* seeds. *Eurasian Chem Commun* **3**(4):244–256 (2021). https://doi.org/10.22034/ecc.2021.273055.1137.
80. Arumugam EVS, Analysis of bioactive constituents from organic crude ethanol extracts from the local medicinal Plant of *Cassytha filiformis* L. (Lauraceae) by gas chromatography-mass spectrometry. *Int J Pharm Sci Rev Res* **28**(39):220–223 (2014).
81. Abdullah BM, Mehdi MAH, Khan AR and Pathan JM, Gas chromatography-mass spectrometry (GC-MS) analysis of ajwain (*Trachyspermum ammi*) seed extract. *Int J Pharm Qual Assur* **11**(2):231–234 (2020). https://doi.org/10.25258/ijpqa.11.2.6.
82. Igwe OU and Abii T, Characterization of bioactive Sesquiterpenes, organic acids and their derivatives from the leaves of *Psidium guajava* Linn. *Int Res J Pure Appl Chem* **4**(4):456–467 (2014). https://doi.org/10.9734/IRJPAC/2014/8592.
83. Mmuta EC, Ogbuagu JO, Arinze RU, Aniecheonwu EA, Agusiobo KT, Echekoba CA *et al*., Phytochemical analysis and GC-MS based bioactive compounds determination of 60 days Nigerian *Vigna radiata* aqueous root extract. *J Pharm Biopharm Res* **6**(1):468–476 (2024). https://doi.org/10.25082/JPBR.2024.01.003.
84. Shoge M and Amusan TO, Phytochemical, antidiarrhoeal activity, isolation and characterisation of 11-Octadecenoic acid, methyl ester isolated from the seeds of *Acacia nilotica* Linn. *J Biotechnol Immunol* **2**(1):229–235 (2020).
85. Kwaji A and Martin I, Isolation and partial characterization of 11-Octadecenoic acid methyl Ester and Bis (2-ethylhexyl) phthalate from *Celtis integrifolia* lam. *Asian Plant Res J* **12**(5):48–55 (2024). https://doi.org/10.9734/aprj/2024/v12i5269.
86. Ayoola AA, Ekunseitan DA, Muhammad SB, Oguntoye MA and Adejola YA, Phytochemicals analysis and GC-MS determination of Ethanolic extracts of *Azadirachta indica* and *Mangifera indica* stem bark and their biological potentials. *Pac J Sci Technol* **21**(1):219–229 (2020).
87. Jethro Y, Oyewale AO and Abdulkadir IJ, Determination of fatty acid methyl ester composition of mango (*Mangifera indica* L.) kernel oil and tribological properties of its triesters. *J Appl Sci Environ Manag* **27**(7):1435–1444 (2023). https://doi.org/10.4314/jasem.v27i7.16.
88. Sutrisno K, Oktaviana L and Wijaya HW, Characterization of some marine fish oils and identification of their fatty acid content at Indonesia's East Java oceans. *AIP Conf Proc* **2588**:1–5 (2023). https://doi.org/10.1063/5.0111970.
89. Gomaa A, Desoukey S and Kamel M, GC-MS analysis of volatile oil and fatty acids composition of *Abutilon hirtum* (lam.) sweet leaves. *J Adv Biomedical Pharm Sci* **4**(3):119–123 (2021). https://doi.org/10.21608/jabps.2021.57135.1116.
90. Kalogiouri NP, Manousi N, Mourtzinos I, Rosenberg E and Zachariadis GA, A rapid GC-FID method for the determination of fatty acids in walnut oils and their use as markers in authenticity studies. *Food Anal Methods* **15**(3):761–771 (2022). https://doi.org/10.1007/s12161-021-02157-3.
91. Liu Y, Xu L, Hu L, Chen D, Yu L, Li X *et al*., Stearic acid methyl ester promotes migration of mesenchymal stem cells and accelerates cartilage defect repair. *J Orthop Translat* **22**:81–91 (2020). https://doi.org/10.1016/j.jot.2019.09.008.
92. Patti A, Lecocq H, Serghei A, Acierno D and Cassagnau P, The universal usefulness of stearic acid as surface modifier: Applications to the polymer formulations and composite processing. *J Ind Eng Chem* **96**:1–33 (2021). https://doi.org/10.1016/j.jiec.2021.01.024.
93. García-Salinas S, Elizondo-Castillo H, Arruebo M, Mendoza G and Irusta S, Evaluation of the antimicrobial activity and cytotoxicity of different components of natural origin present in essential oils. *Molecules* **23**(6):1399 (2018). https://doi.org/10.3390/molecules23061399.
94. ISO 10993-5:2009, Biological evaluation of medical devices – Part 5: Tests for in vitro cytotoxicity (2009).
95. Marinas IC, Oprea E, Buleandra M, Badea IA, Tihauan BM, Marutescu L *et al*., Chemical composition, antipathogenic and cytotoxic activity of the essential oil extracted from *Amorpha fruticosa* fruits. *Molecules* **26**(11):3146–3163 (2021). https://doi.org/10.3390/molecules26113146.
96. Javanshir A, Karimi E, Maragheh AD and Tabrizi MH, The antioxidant and anticancer potential of *Ricinus communis* L. essential oil nanoemulsions. *J Food Meas Charact* **14**(3):1356–1365 (2020). https://doi.org/10.1007/s11694-020-00385-5.
97. Niu H, Li X, Yang A, Jin Z, Wang X, Wang Q *et al*., Cycloartenol exerts anti-proliferative effects on glioma U87 cells via induction of cell cycle arrest and p38 MAPK-mediated apoptosis. *J BUON* **23**(6):1840–1845 (2018).
98. Zaghlol AA, Kandil ZA, Yousif MF, el-Dine RS and Elkady WM, Unveiling the anti-cancer potential of *Euphorbia greenwayi*: Cytotoxicity, cell migration, and identification of its chemical constituents. *Futur J Pharm Sci* **10**:24 (2024). https://doi.org/10.1186/s43094-024-00599-0.
99. Leyva-Peralta AA, Robles-Zepeda EE, Garibay-Escobar A, Ruiz-Bustos E, Alvarez-Berber PP and Gálvez-Ruiz CC, In vitro anti-proliferative activity of *Argemone gracilenta* and identification of some active components. *BMC Complement Altern Med* **15**(1):1–7 (2015). https://doi.org/10.1186/s12906-015-0532-8.
100. Mota NSRS, Kviecinski MR, Zeferino RC, de Oliveira DA, Bretanha LC, Ferreira SRS *et al*., *In vivo* antitumor activity of by-products of *Passiflora edulis f. flavicarpa* Deg. rich in medium and long chain fatty acids evaluated through oxidative stress markers, cell cycle arrest, and apoptosis induction. *Food Chem Toxicol* **118**:557–565 (2018). https://doi.org/10.1016/j.fct.2018.06.010.
101. Zafaryab M, Fakhri KU, Khan MA, Hajela K and Rizvi MMA, In vitro assessment of cytotoxic and apoptotic potential of palmitic acid for breast cancer treatment. *Int J Life Sci Res* **7**(1):166–174 (2019). https://doi.org/10.13140/RG.2.2.33419.13606.

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102. Djeghim H, Benouchenne D, Mokrani EH, Alsaeedi H, Cornu D, Bechelany M *et al*., Antioxidant, anti-Alzheimer's, anticancer, and cytotoxic properties of peanut oil: In vitro, in silico, and GC-MS analysis. *Front Chem* **12**:1487084 (2024). https://doi.org/10.3389/fchem.2024.1487084.



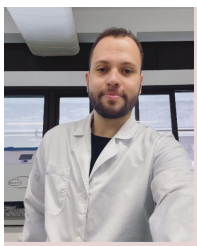
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