Toxicology Reports 13 (2024) 101693

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

In vitro cytotoxicity and genotoxicity assessment of methanolic extracts of vanillas from Brazilian biodiversity with commercial potential

Natália Gonçalves Ribeiro Araujo [a,1], Carlos Fernando Araujo-Lima [a,b,*,2], Renatha Tavares de Oliveira [c,3], Andrea Furtado Macedo [c,4], Israel Felzenszwalb [a,5]

[a] Laboratory of Environmental Mutagenesis (LABMUT), University of Rio de Janeiro State (UERJ), Rio de Janeiro, RJ, Brazil
[b] Laboratory of Pharmaceutical and Technological Innovation, Federal University of Rio de Janeiro State (UNIRIO), Rio de Janeiro, RJ, Brazil
[c] Laboratory of Integrated Plant Biology (LIBV) - Federal University of Rio de Janeiro State (UNIRIO), Rio de Janeiro, RJ, Brazil

ARTICLE INFO

Handling Editor: Prof. L.H. Lash

Keywords:
Vanilla
Mutagenicity
Bioprospection
Genotoxicity

ABSTRACT

The *Vanilla* genus is crucial for global production in food, perfume, and pharmaceutical industries. However, exploitation threatens some species, leading to extinction. Traditional communities use vanilla for medicinal purposes, and there are species like *Vanilla chamissonis* Klotzsch and *Vanilla bahiana* Hoehne with potential to occupy the market. For this, methanolic extraction of these two mentioned species was conducted alongside *Vanilla planifolia*. Analyzes of the cell viability, mutagenic and genotoxic potential were performed. In the Ames test, the assays were performed with concentrations from 0.5 and 5000 μg/ml and on five strains. Only *Vanilla planifolia* exhibited mutagenicity at the highest concentration in the TA98 strain. Viability tests were performed within a dose range of 0.05–5000 μg/ml and 24, 48, and 72-hour exposures. It was possible to observe a reduction in cell viability observed only at the highest concentration, for all three species and both cell types tested. Genotoxicity induction by the extracts was assessed at concentrations from 0.5 to 500 μg/ml through the cytokinesis-block micronucleus assay. No genotoxic damage or reduction in the Nucleus Division Index (NDI). The study found no mutagenicity, cytotoxicity, or genotoxicity in the species tested, indicating potential human use for food or pharmaceutical purposes.

1. Introduction

Vanilla, a spice renowned for its characteristic aroma and flavor, holds immense importance in the world economy. Its production primarily involves extracting from certain species of the *Vanilla* genus, which share critical molecules in their composition that give Vanilla its distinctive flavor, such as vanillin [1]. The main species currently in the market are *Vanilla planifolia* Jacks. *ex* Andrews, *Vanilla* × *tahitensis* J. W. Moore, and *Vanilla pompona* Schiede, with cultivation concentrated mainly in the Madagascar region and to a lesser extent elsewhere. This underscores the economic value of vanilla and the significance of our work in preserving and utilizing this precious resource.

As previously mentioned, one of the main factors hindering large-scale cultivation of the *Vanilla* genus is its difficult pollination. Although they managed to overcome this obstacle, production is still vanillin, which increases the use of synthetic vanillin, which is insufficient to meet world demand and increases synthetic vanillin use [2]. In addition, other issues led to the "vanilla crisis" during the 21st century, when the price of a kilo of vanilla rose exorbitantly, even surpassing the cost of a kilo of silver [3,4].

Brazil has rich biodiversity and naturally occurring vanilla species, such as *Vanilla bahiana* and *Vanilla chamissonis*, which have yet to be

* Correspondence to: Laboratory of Pharmaceutical and Technological Innovation, Federal University of Rio de Janeiro State (UNIRIO), R. Frei Caneca, 94 - Centro, Rio de Janeiro, RJ 20211-010, Brazil.
E-mail addresses: nataliagra100@gmail.com (N.G.R. Araujo), biomed.carlos@gmail.com (C.F. Araujo-Lima), renathat.oliveira@edu.unirio.br (R.T. Oliveira), andrea.macedo@unirio.br (A.F. Macedo), uerj.felzen@gmail.com (I. Felzenszwalb).
[1] Orcid ID: 0000–0002-4822–6194
[2] Orcid ID: 0000–0002-9278–4441
[3] Orcid ID: 0000–0002-5099–6032
[4] Orcid ID: 0000–0002-1791–7339
[5] Orcid ID: 0000–0003-1677–197X

https://doi.org/10.1016/j.toxrep.2024.101693
Received 10 June 2024; Accepted 11 July 2024
Available online 14 July 2024

explored and have significant economic potential. These species were subjected to phytochemical analysis, revealing vanillin in their composition and other compounds [5,6] that together promote a sensory experience similar to the known flavor described as vanilla, conferring organoleptic properties identical to those of the main commercialized species. Therefore, it is essential to investigate these species since they have great potential for local bioprospecting [5].

In addition to its food and aromatic uses, vanilla has traditionally been used for medicinal purposes in various cultures [7]. Recognizing the centuries-old use of the genus, several studies have been conducted considering the importance of investigating the therapeutic potential of the spice, which, to this day, has been associated with relevant therapeutic effects [8]. The studies involve the evaluation of different species as well as their main constituent, vanillin. So far, results show vanillin's antibiotic properties, as well as its potential agent in the treatment of sickle cell anemia [9,10]. Anti-inflammatory effects on the nervous system have also been observed, which is important in acting on neuroinflammatory pathologies [11]. Most studies focus on the anticarcinogenic activity of vanillin, as observed in colorectal cancer (HT-29), hepatocarcinoma (HepG2), and neuroblastoma (SH-SY5Y) cells, in addition to the antioxidant, cardioprotective, and antiviral properties described [12–14].

The medicinal properties of plants are fundamental, not only in the context of health care but also in pharmaceutical development. In contrast, natural products may present risks, mainly when not adequately evaluated for their potential toxicity [15,16]. Conducting tests to examine their phytochemical composition and bioactivity and investigate their genotoxic potential identifies compounds that can threaten health and reveal therapeutic properties, such as antimutagenic, anticarcinogenic, and antigenotoxic properties. These properties are of great interest in the context of medical applications [16–18].

2. Materials and methods

2.1. Vanilla extraction

The specimens evaluated in this study were sourced from distinct locations: *V. bahiana* was collected from an individual within the Natural Monument from the Morro do Pão de Açúcar at Urca, Rio de Janeiro, Brazil. *V. chamissonis* was obtained from an individual within the Municipal Natural Park of Grumari, Rio de Janeiro, Brazil. Lastly, *V. planifolia* was obtained from an individual located in the Orchidarium of the Rio de Janeiro Botanical Garden.

The methanolic extraction was carried out using three fruits: *Vanilla planifolia*, *Vanilla chamissonis*, and *Vanilla bahiana*. The fruits underwent drying and maceration, followed by enzymatic extraction to accelerate the curing process [5]. For this, 500 mg of the samples were placed in 3 ml of citrate-phosphate buffer (0.05 M; pH 5), homogenized, and then subjected to a water bath at 37ºC. Subsequently, 1 ml of the ß-glucosidase enzyme solution (49290/SIGMA), previously prepared with almond ß-glucosidase and citrate-phosphate buffer at a ratio of 1 mg/ml, was added. The mixture was incubated for 4 hours at 37ºC with constant agitation. Following incubation, the samples were frozen using liquid N2 to preserve their integrity. Subsequently, the samples were lyophilized until completely dry.

Then, the methanolic extraction was conducted. Methanol (5 ml) was added to the Falcon tubes containing the enzymatic extraction product and mixed for 2 minutes on a vortex. The samples were then subjected to probe sonication for 8 minutes at 20 % power, supported on a stand with ice and water to prevent heating. After sonication, the samples were centrifuged (10000 x g at 4ºC for 10 min), and the supernatant was transferred to a 15 ml Falcon tube and stored in the freezer. This extraction procedure was repeated two more times, and at the end of the process, the samples were dried using a speed-vac.

2.2. Mutagenicity test

The mutagenic potential of the Vanilla extracts was analyzed through a reverse mutation test; the Ames test was conducted on strains of Salmonella enterica serovar Typhimurium (S. typhimurium) TA97a, TA98, TA100, TA102, and TA1535. The strains were chosen following the OECD to analyze the most significant amount of mutation induction possible with the model [19]. The test utilized the pre-incubation method and was performed in the absence and presence of a metabolic activation system (4 % S9 mix, Aroclor-pre-induced, sourced from Moltox Inc., USA) [20]. The positive control for the assays conducted without metabolic activation were 4-nitroquinoline 1-oxide (4-NQO) for TA97a (2 μg/plate) and TA98 (1 μg/plate); sodium azide (SA) for TA100 and TA1535 (5 μg/plate) and mitomycin C (MMC) for TA104. With metabolic activation, the positive control was 2-amineanthracene (2-AA) for all the strains in different concentrations: TA97a and TA98 at 2 μg/plate; TA100, TA102, and TA1535 at 5 μg/plate.

For the experiments without metabolic activation, 0.5 ml of 0.1 mol L^{-1} sodium-phosphate buffer (with a pH of 7.4) was added to the test tube, in the experiments with metabolic activation, 0.5 ml of the S9 mix was used instead. Then, 0.1 ml of culture medium (with a cell density of 2×10^9 cells/ml) was added along with 0.1 ml of each concentration of Vanilla tested. The tubes were incubated for 20 minutes at 37°C with agitation. After the incubation period, 2 ml of Top Agar, consisting of agar with 10 % HB (Histidine and Biotin solution), was added. The plates were then incubated for 72 hours at 37°C, and finally, the revertant colonies were counted.

The tester strains were assayed in triplicate, and the count of revertant colonies was recorded for each strain and treatment group. Positivity criteria were established based on the observation of a concentration-dependent increase in the average number of revertant colonies within each treated group, achieving a minimum twofold elevation compared to the count observed in the negative control group. All the experiments were repeated at least twice. Statistical differences between the groups were analyzed by a one-way ANOVA ($P < 0.05$) and Tukey's post-hoc test.

2.3. Eukaryotic Cell viability

Two eukaryotic cell lines were utilized for the cell viability analysis: human hepatocellular carcinoma cells, HepG2, and mouse liver fibroblasts, FC3H. HepG2 cells were obtained from the American Type Culture Collection (ATCC, Manassas VA, USA, #HB-8065), while the FC3H lineage was acquired from the Rio de Janeiro Cell Bank (BCRJ, Rio de Janeiro, BR, #BCRJ 0082). Following supplier specifications, both hepatic tissue cell lines were maintained at the Environmental Mutagenesis Laboratory - UERJ. DMEM with 10 % FBS was used for HepG2, and DMEM High Glucose with 10 % FBS for FC3H. Cell viability was assessed after exposure to methanolic extracts of Vanilla for 24, 48, and 72 hours to determine their potential cytotoxicity-inducing effects.

To conduct the assay, cells were initially plated in 96-well plates at a density of 2×10^4 cells/well, with 3 wells allocated for each tested extract concentration and at least 3 wells reserved for assay controls. Since the assay analyses three-time points, three plates were prepared. Following cell plating on the first day and cell adhesion, the wells of all three plates were washed with PBS and 100 μl of dilutions of the different extract concentrations. The extracts were diluted in DMSO, so it was important to ensure that the DMSO, which is cytotoxic, was present at a concentration lower than 1 % in the wells. Serial dilutions were prepared using the appropriate media for each cell type. The prepared concentrations were: 5000 μg/ml, 500 μg/ml, 50 μg/ml, 5 μg/ml, 0.5 μg/ml, and 0.05 μg/ml. In addition to the extract samples, assay controls were performed, including three wells with medium only, three with Triton X-100 2 %, and three with the vehicle used to dilute the samples (DMSO 1 %). After treatments, the plates were incubated in a chamber under the conditions described earlier for 24, 48, or 72 hours.

Following each incubation period, the wells were washed, and 100 µl of a 2 % WST solution was added to each well, including the three wells without cells. The plates were then incubated for 2 hours, after which absorbance readings were taken at 440 nm using a microplate reader configured for spectrum detection. The assay was performed in triplicate and repeated twice. The obtained results were exported for future statistical analysis. Linear regression was performed using the obtained values, with viability control consisting of cells receiving only medium and non-viability controls being wells without cells. From this, the cell viability curve was calculated.

2.4. Cytokinesis-block micronucleus assay (MNvit)

Cells were plated onto a 24-well plate containing sterile coverslips and seeded at a density of 2×10^4 cells/well, each with a final volume of 1 ml. The plates were then incubated at 37°C and 5 % CO_2 overnight to allow cell adhesion to the coverslips. On the following day, the treatment was performed. Initially, the wells were washed with PBS, and four concentrations of methanolic extracts from each species were added for testing: 500 µg/ml, 50 µg/ml, 5 µg/ml, and 0.5 µg/ml. Additionally, cells were treated with Cyclophosphamide (CPA) at 60 µM (positive control), 1 % DMSO (negative control), and medium only. Once again, the cells were incubated at 37°C and 5 % CO2 for 24 hours.

The cells were washed with PBS, and 1 ml of a Cytochalasin B solution was added to each well at a 3 µg/ml concentration. The plates were incubated at 37°C and 5 % CO_2 for 24 hours. On the last day of the assay, the medium was removed. Each coverslip was fixed by adding a 3:1 solution of cold methanol and acetic acid. 1 ml was added to each well, and the solution was allowed to be fixed for 30 minutes. Subsequently, the solution was removed, and the wells were washed with water, which was then discarded. Next, 1 ml of Giemsa 1:4 was added to each well and left to stain for 2 hours. Afterward, each well was rewashed with water, and the slides were prepared using Entellan to affix the coverslips onto their respective slides, which were previously identified.

To analyze results, at least 2000 binucleated cells per concentration were counted, considering observed cellular events such as bridges, buds, micronuclei, and mononucleated, trinucleated, and multinucleated cells. The Nuclear Division Index (NDI) and micronucleus count were calculated. The results were analyzed using one-way ANOVA and Tukey's post-hoc test to assess statistically significant outcomes.

3. Results

3.1. Mutagenesis induction analysis

Mutagenicity induction was detected only under specific conditions and in a single species, *Vanilla planifolia*, in the TA98 strain without metabolic activation, as depicted in Fig. 1. A dose-response curve can be observed, with mutagenicity induction confirmed at 4000 and 5000 µg/plate concentrations. Except for these conditions, no further mutagenicity induction was observed in the tested strains and analyzed species, as illustrated in Fig. 1.

3.2. Eukaryotic cell viability

Through the WST-1 assay, the effect of methanolic extracts on cell viability was assessed. Fig. 2 demonstrates that the extracts of *V. planifolia* and *V. bahiana* significantly reduced cell viability, but only at the highest concentration of 5000 µg/ml in HepG2 cells across all tested time points. Similar observations were made in FC3H cells, as shown in Fig. 2, although *V. bahiana* induced decreased viability, while *V. planifolia* declined only at the highest concentration. Additionally, *V. chamissonis* also reduced cell viability in FC3H cells, but solely at the 24-hour treatment and in a concentration-dependent manner.

3.3. Cytokinesis-block micronucleus assay

In the Cytokinesis-Block Micronucleus Assay, as Fig. 3 shows, none of the evaluated species were able to induce genotoxic damage through the formation of micronuclei, nor did they delay the cell cycle, as indicated by the Nuclear Division Index (NDI) calculations.

4. Discussion

Various therapeutic effects have been attributed to *Vanilla* over time, initially through folk medicine and currently through research demonstrating the genus's significant potential, including in clinical trials. In these, the exposure to vanillin has been found to reduce the occurrence of breathing pauses and prevent slow heart rate, potentially by activating the olfactory nerve and increasing orbitofrontal blood flow, in newborns and adults [21,22]. Recognizing this emphasizes the importance of understanding the safety of this genus, especially of species not yet popularly used and that have little explored.

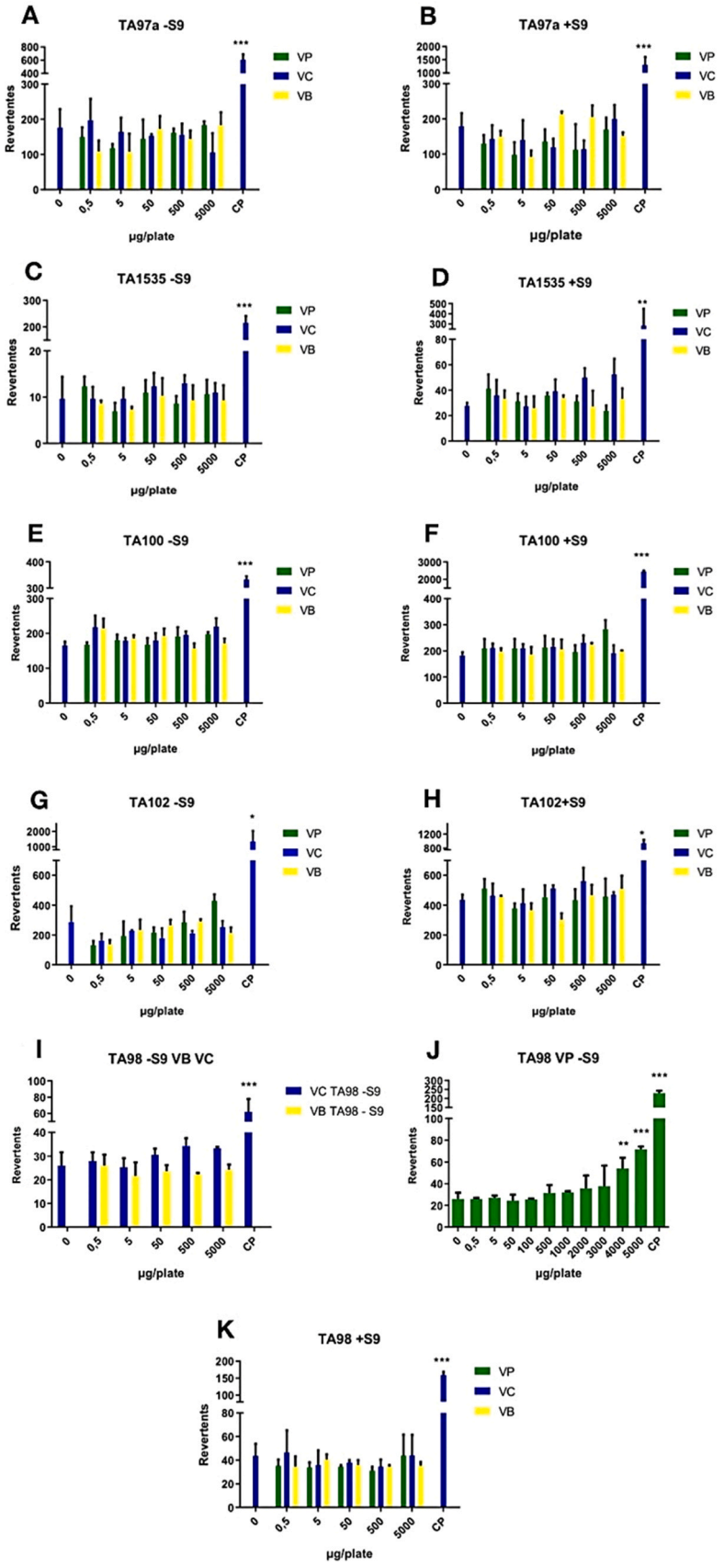
Synthetic vanillin is widely used in the food industry. However, it has been observed that this and other artificial ingredients could induce *in vivo* toxicity in mice [23], including genotoxicity. Although many bioactives are found in natural products, this does not guarantee their safety. Vanillin is a secondary metabolite in *Vanilla spp.* species have a great economic interest, both for their flavor and characteristic fragrance, and are of great interest to the food and perfumery industries [24]. For this reason, species with higher quantities of vanillin and other molecules contributing to the sensory experience of vanilla, such as vanillic acid and *p*-hydroxybenzaldehyde, have made this genus so popular and consumed for so long, remaining extremely important for large companies in the food industry [25,26].

Among the analyzed species with the potential to meet this market demand, the induction of mutagenicity or cytotoxicity was observed only at higher concentrations to which the cells or bacteria were exposed. This indicates that the species' methanolic extracts are safe in assays evaluating their cytotoxic and mutagenic potential.

The bacterial reverse mutation assay, the Ames test, assessed the mutagenic potential of the compounds analyzed. The only sample capable of inducing mutation was the one of greater commercial relevance, *Vanilla planifolia*, and only at the highest concentrations of 4000 and 5000 µg/plate and only in TA98. Still, the commercial potential species investigated in this study could not induce mutations, which is promising for their use.

The *V. planifolia* and *Vanilla* × *tahitensis* extracts are recognized as safe by the FDA (U.S. Food and Drugs Administration) [27]. Human exposure to these extracts is usually in a low dose since they are mainly used as spices and natural seasoning; e.g., most baking recipes use a teaspoon of vanilla extract, which corresponds to approximately 4 g [28]. Analyzing one of the most important molecules, vanillin, *in vivo,* assays gave rats vanillin doses of 150 and 300 mg/kg for 14 weeks did not exhibit any signs of toxicity [29]. Another study demonstrated that rats fed with vanillin in their diet at 20 g/kg for 1 year and 50 g/kg for 2 years showed no changes in growth or blood composition [30]. Similar assays [31,32] associated with these findings show that vanilla components are not toxic when consumed at approved levels in food [33].

The induction of mutation at higher concentrations by *V. planifolia* may be justified by the presence of phenolic contents and other organic compounds in the extract that have antioxidant potential and, as such, can generate a saturation effect inherent to biological systems. Thus, compounds that usually scavenge free radicals also have antioxidant action and can behave as pro-oxidants, as observed in studies with other plant extracts with high antioxidant content [34,35]. As mentioned earlier, the complex mixture of joint activity causes interaction with different cellular sites and targets simultaneously. Plant extracts can generate joint activity and interaction with cellular sites and targets. This mechanism of action found in natural products derived from plants can trigger adverse effects when in contact with other organisms [36,



(caption on next page)

Fig. 1. Revertant colonies in the Ames test with (+S9) and without (-S9) metabolic activation of the strains TA97a, TA198, TA100, TA102 and TA1535. In A, revertants of TA97a for the assay without metabolic activation (-S9) of the species *V. planifolia* (VP), *V. chamissonis* (VC), and *V. bahiana* (VB) are shown. In B, revertants of TA97a in the assay with metabolic activation (+S9) of the three species are depicted. In C, revertants of TA1535 in the assay with metabolic activation (-S9) of the three species are illustrated, while in D, the results in TA1535 with the three species of Vanilla and metabolic activation (+S9) are presented. Graphic E and F depict results for TA100, with E and F showing assays without and with metabolic activation, respectively. In G, revertants of TA102 in the assay with metabolic activation (-S9) of the three species are illustrated. In contrast, in H, the results in TA102 with the three species of Vanilla and metabolic activation (+S9) are presented. In I, the results are for the assay with TA98 without metabolic activation (-S9) of the species *V. chamissonis* (VC) and *V. bahiana* (VB). In J, the assay results showed TA98 without metabolic activation (-S9) of the species *V. planifolia* (VP), with additional concentrations tested. In K, the results with TA98 with all three species with metabolic activation (+S9) are presented.

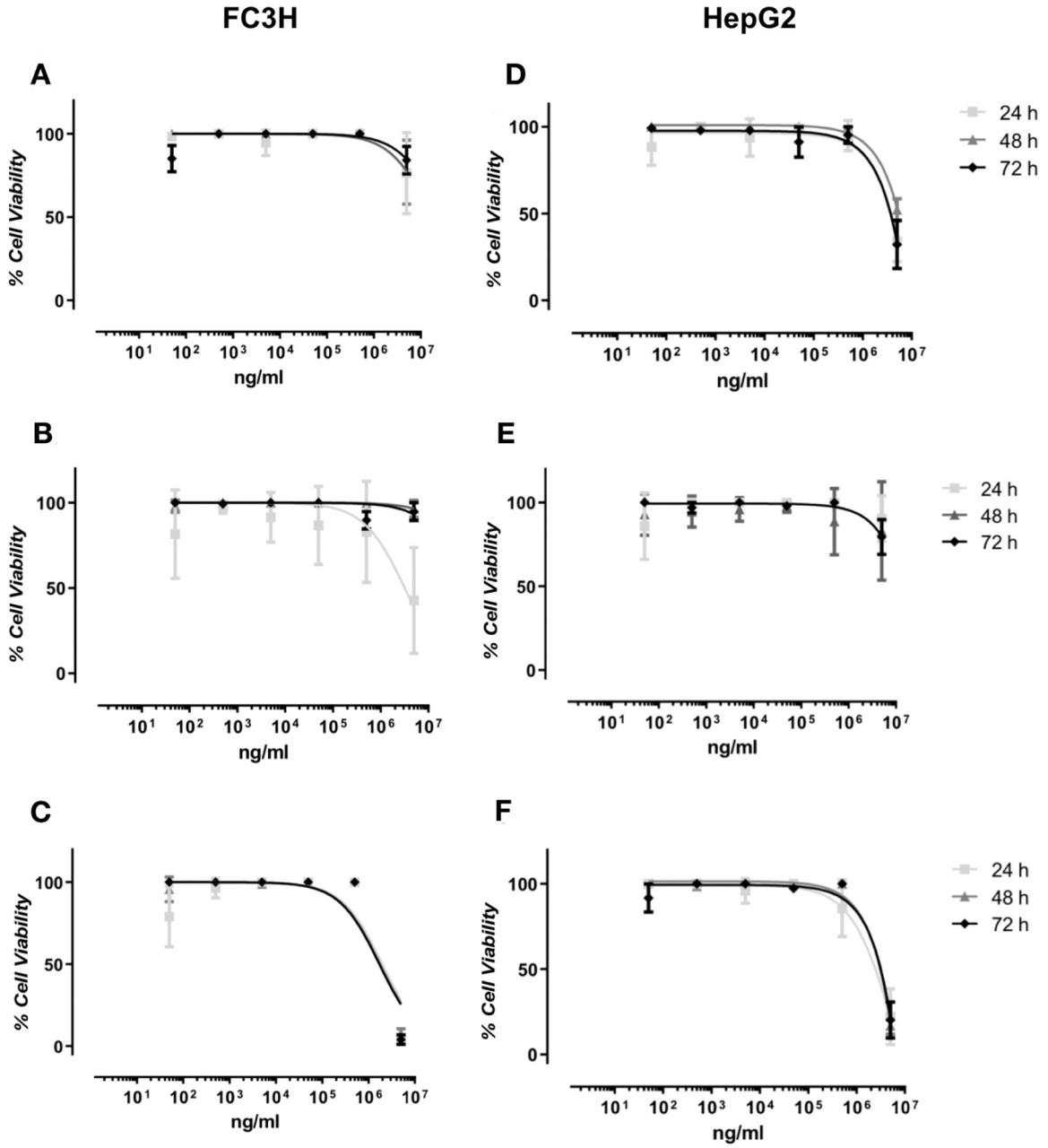


Fig. 2. (A) Viability of FC3H cells after 24, 48, and 72 hours of treatment with *V. planifolia*. (B) Viability of FC3H cells after 24, 48, and 72 hours of treatment with *V. chamissonis*. (C) Viability of FC3H cells after 24, 48, and 72 hours of treatment with *V. bahiana*. (D) Viability of HepG2 cells after 24, 48, and 72 hours of treatment with *V. planifolia*. (E) Viability of HepG2 cells after 24, 48, and 72 hours of treatment with *V. chamissonis*. (F) Viability of HepG2 cells after 24, 48, and 72 hours of treatment with *V. bahiana*.

37]. Nevertheless, the analyzed doses are much higher than the normal human intake of vanilla.

Previous studies have shown *V. bahiana* to be a potential spice market species, as it has been shown to express some of the most essential enzymes in the biosynthesis of vanilla flavoring compounds [38]. Using Liquid Chromatography-Mass Spectrometry (LC-MS/MS) and chemometric techniques, it was observed that both *V. bahiana* and *V. chamissonis* have compounds related to the characteristic flavor of

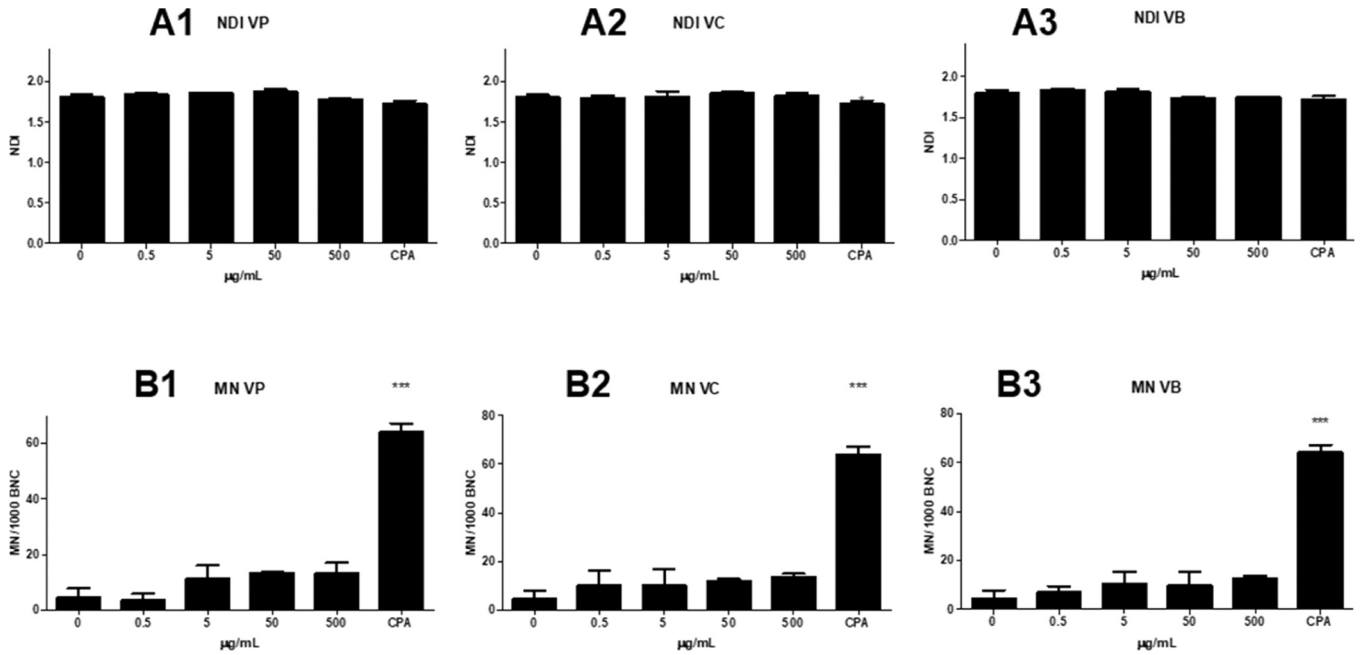


Fig. 3. Nuclear Division Index (NDI) and Micronucleus count. (A1) NDI of HepG2 cells treated with *V. planifolia*. (A2) NDI of HepG2 cells treated with *V. chamissonis*. (A3) NDI of HepG2 cells treated with *V. bahiana*. (B1) Micronucleus induction count in HepG2 cells treated with *V. planifolia*. (B2) Micronucleus induction count in HepG2 cells treated with *V. chamissonis*. (B3) Micronucleus induction count in HepG2 cells treated with *V. bahiana*.

vanilla, which is associated with commercial species, such as vanillin and *p*-hydroxybenzaldehyde [5].

Vanillin has demonstrated potential in therapeutic applications, such as neuroprotection, antiviral, cardioprotection, anti-inflammatory, and nephroprotection [8]. In addition, antigenotoxic properties and anti-mutagenic activity have also been reported for vanillin by reducing spontaneous mutations in both bacteria and mammalian cells [39,40]. Another compound in the genus is vanillic acid, which is also found in other plants. This acid and cinnamic acid has been shown to reduce DNA damage [41]. Vanillin has already been identified in all three species; therefore, it is important to investigate their therapeutic potential, especially the DNA protection activities these species can offer. As mentioned, a vanillin formulation used in the food market has shown the potential to induce genotoxicity by micronucleus formation. Still, the same was not observed for the extracts of the different species analyzed, nor the induction of cytochalasin [23].

The economically most relevant species, *V. planifolia*, has already shown antiproliferative activities, as seen in the ethanolic extract of *V. planifolia* leaf in squamous carcinoma and breast cancer cells [42,43]. Positive results of vanillin in inducing apoptosis in colorectal cancer cells and inhibiting the growth of these cells have also been obtained [44], in addition to inducing apoptosis of hepatocarcinoma and neuroblastoma cells [45].

The species *V. bahiana* and *V. chamissonis* have been little explored in this context. Considering that many of the compounds found in *V. planifolia* have also been observed in them [5], it is expected that similar therapeutic effects may be found, with potentially lesser or greater activities, given that the association between the molecules present in complex mixtures can generate different interactions. Furthermore, the identification of compounds present in these orchids also allows for the analysis of their content and evaluation of whether the medicinal properties are attributed to one or more molecules. Synergistic events may still occur, which is common in these complex mixtures where different associated compounds generate a more significant result than the sum of their parts, enhancing the pharmaceutical potential of the extracts [46,47].

The *Vanilla* species *V. planifolia*, *V. bahiana*, and *V. chamissonis* hold significant economic promise due to their flavor, making *Vanilla* a key spice with increasingly therapeutic potential. Our study assessed whether methanolic extracts of these species could induce genotoxicity or cytotoxicity. Testing with the Bacterial Reverse Mutation Test and the Cell Viability Assay revealed mutation or cytotoxicity induction only at the highest doses tested: 4000 and 5000 µg/plate in the prokaryotic model (TA98 -S9) with *V. planifolia* and 5000 µg/ml in the eukaryotic model with all three species. No genotoxicity induction by Micronucleus formation was observed. Thus, these Brazilian species show promise for evaluating these new compounds for human use in the food market and pharmaceuticals.

CRediT authorship contribution statement

Carlos Fernando Araújo Lima: Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing. **Natália Gonçalves Ribeiro Araujo:** Data curation, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Renatha Tavares de Oliveira:** Investigation, Methodology, Validation. **Andrea Furtado Macedo:** Conceptualization, Project administration, Resources, Supervision, Visualization, Writing – review & editing. **Israel Felzenszwalb:** Conceptualization, Formal analysis, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by UNIRIO [INOVA 01/2021, INOVA 02/2022, and PPQ 03/2022 – AFM; PPQ 03/2022 and PPInst 02/2022 – CFA-L], Coordination of Superior Level Staff Improvement (CAPES)

(Code 0001); National Council for Scientific and Technological Development – CNPq [404700/2023–3 – AFM; 408259/2023–0 – CFA-L; 302345/2017–5 – IF; 165757/2021–3 - NGRA] and The Carlos Chagas Filho Foundation for Research Support of the State of Rio de Janeiro (FAPERJ) [E-26/202.759/2017 (IF); E-26/210.096/2023 (CFA-L and IF)]. The Carlos Chagas Filho Foundation for Research Support of the State of Rio de Janeiro FAPERJ N°13/2023 – Auxílio Básico à Pesquisa (APQ1) – 2023. The authors thank CAPES [RTO, 001]. We also want to thank the Botanic Garden Research Institute of Rio de Janeiro, especially Dr. Delfina de Araujo, for donating the fruits of *Vanilla planifolia*.

References

[1] D.S. Correll, Vanilla- Its Botany, History, Cultivation and Economic Import, Econ. Bot. (1948) 291–292.
[2] A. Gratzfeld-Huesgen, Analysis of natural and artificial vanilla preparations, Agil. Technol. (2011).
[3] R.J. Brownell, Fair Trade - The Future of Vanilla? Handb. Vanilla Sci. Technol. (2010) 107–116, https://doi.org/10.1002/9781444329353.ch7.
[4] G.A. Martău, L.F. Călinoiu, D.C. Vodnar, Bio-vanillin: Towards a sustainable industrial production, Trends Food Sci. Technol. 109 (2021) 579–592, https://doi.org/10.1016/j.tifs.2021.01.059.
[5] J.P. da, S. Oliveira, R. Garrett, M.G.B. Koblitz, A.F. Macedo, Vanilla flavor: Species from the Atlantic forest as natural alternatives, Food Chem. 375 (2022) 131891, https://doi.org/10.1016/j.foodchem.2021.131891.
[6] R.T. de Oliveira, J.P. da Silva Oliveira, A.L.M. da Silva, E.K.C. Dantas, M.G. B. Koblitz, M.L. Bello, I. Felzenszwalb, C.F. Araújo-Lima, A.F. Macedo, Vanilla from Brazilian Atlantic Forest: In vitro and in silico toxicity assessment and high-resolution metabolomic analysis of Vanilla spp. ethanolic extracts, Food Chem. (2024) 139948, https://doi.org/10.1016/j.foodchem.2024.139948.
[7] J.D. Bythrow, Vanilla as a Medicinal Plant, Semin. Integr. Med. 3 (2005) 129–131, https://doi.org/10.1016/j.sigm.2006.03.001.
[8] S.S. Arya, J.E. Rookes, D.M. Cahill, S.K. Lenka, Vanillin: a review on the therapeutic prospects of a popular flavouring molecule, Adv. Tradit. Med. 21 (2021) 415–431, https://doi.org/10.1007/s13596-020-00531-w.
[9] D.J. Abraham, A.S. Mehanna, F.C. Wireko, J. Whitney, R.P. Thomas, E.P. Orringer, Vanillin, a potential agent for the treatment of sickle cell anemia, Blood 77 (1991) 1334–1341. ⟨http://www.ncbi.nlm.nih.gov/pubmed/2001455⟩.
[10] N.A. Maisch, S. Bereswill, M.M. Heimesaat, Antibacterial effects of vanilla ingredients provide novel treatment options for infections with multidrug-resistant bacteria – A recent literature review, Eur. J. Microbiol. Immunol. 12 (2022) 53–62, https://doi.org/10.1556/1886.2022.00015.
[11] M.E. Kim, J.Y. Na, Y.D. Park, J.S. Lee, Anti-Neuroinflammatory Effects of Vanillin Through the Regulation of Inflammatory Factors and NF-κB Signaling in LPS-Stimulated Microglia, Appl. Biochem. Biotechnol. 187 (2019) 884–893, https://doi.org/10.1007/s12010-018-2857-5.
[12] A. Tai, T. Sawano, F. Yazama, H. Ito, Evaluation of antioxidant activity of vanillin by using multiple antioxidant assays, Biochim. Biophys. Acta - Gen. Subj. 1810 (2011) 170–177, https://doi.org/10.1016/j.bbagen.2010.11.004.
[13] M. Hariono, N. Abdullah, K.V. Damodaran, E.E. Kamarulzaman, N. Mohamed, S. S. Hassan, S. Shamsuddin, H.A. Wahab, Potential New H1N1 Neuraminidase Inhibitors from Ferulic Acid and Vanillin: Molecular Modelling, Synthesis and in Vitro Assay, Sci. Rep. 6 (2016) 38692, https://doi.org/10.1038/srep38692.
[14] I. Sirangelo, L. Sapio, A. Ragone, S. Naviglio, C. Iannuzzi, D. Barone, A. Giordano, M. Borriello, Vanillin Prevents Doxorubicin-Induced Apoptosis and Oxidative Stress in Rat H9c2 Cardiomyocytes, Nutrients 12 (2020) 2317, https://doi.org/10.3390/nu12082317.
[15] M. Romero-Jiménez, J. Campos-Sánchez, M. Analla, A. Muñoz-Serrano, Á. Alonso-Moraga, Genotoxicity and anti-genotoxicity of some traditional medicinal herbs, Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 585 (2005) 147–155, https://doi.org/10.1016/j.mrgentox.2005.05.004.
[16] M. Akram, M. Riaz, A.W.C. Wadood, A. Hazrat, M. Mukhtiar, S. Ahmad Zakki, M. Daniyal, M.A. Shariati, F. Said Khan, R. Zainab, Medicinal plants with anti-mutagenic potential, Biotechnol. Biotechnol. Equip. 34 (2020) 309–318, https://doi.org/10.1080/13102818.2020.1749527.
[17] G. Sponchiado, M.L. Adam, C.D. Silva, B. Silva Soley, C. De Mello-Sampayo, D. A. Cabrini, C.J. Correr, M.F. Otuki, Quantitative genotoxicity assays for analysis of medicinal plants: A systematic review, J. Ethnopharmacol. 178 (2016) 289–296, https://doi.org/10.1016/j.jep.2015.10.026.
[18] M. Poivre, A. Nachtergael, V. Bunel, O.N. Philippe, P. Duez, Genotoxicity and Carcinogenicity of Herbal Products. Toxicol. Herb. Prod., Springer International Publishing, Cham, 2017, pp. 179–215, https://doi.org/10.1007/978-3-319-43806-1_9.
[19] OECD, Test No. 471: Bacterial Reverse Mutation Test, OECD, 2020, https://doi.org/10.1787/9789264071247-en.
[20] D.M. Maron, B.N. Ames, Revised methods for the Salmonella mutagenicity test, Mutat. Res. Mutagen. Relat. Subj. 113 (1983) 173–215, https://doi.org/10.1016/0165-1161(83)90010-9.
[21] G. Clinica Trials, Clinical Trials, (2023). ⟨https://clinicaltrials.gov/search?intr=vanilla⟩.
[22] M. Edraki, H. Pourpulad, M. Kargar, N. Pishva, N. Zare, H. Montaseri, Olfactory stimulation by vanillin prevents apnea in premature newborn infants, Iran, J. Pediatr. 23 (2013) 261–268.
[23] I.M.S. Sales, J.M. Silva, E.S.R. Moura, F.D.S. Alves, F.C.C. Silva, J.M.C. Sousa, A. P. Peron, Toxicity of synthetic flavorings, nature identical and artificial, to hematopoietic tissue cells of rodents, Braz. J. Biol. 78 (2018) 306–310, https://doi.org/10.1590/1519-6984.07716.
[24] C.C. De Guzman, R.R. Zara, Vanilla, Handb. Herbs Spices Second Ed. 1 (2012) 547–589. https://doi.org/10.1533/9780857095671.547.
[25] J. Ni, F. Tao, H. Du, P. Xu, Mimicking a natural pathway for de novo biosynthesis: Natural vanillin production from accessible carbon sources, Sci. Rep. 5 (1) (2015) 12, https://doi.org/10.1038/srep13670.
[26] L.M.R. Del Egido, Fair trade vanilla market research and import project from Papua New Guinea to Spain, 2021.
[27] FDA, CFR - Code of Federal Regulations Title 21, Spices Other Nat. Seas. Flavorings (1977). ⟨https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=182.10⟩.
[28] P. Sachdev, Health Benefits of Vanilla, WebMD (2022). ⟨https://www.webmd.com/diet/health-benefits-vanilla⟩ (accessed February 21, 2023).
[29] K. Ho, L.S. Yazan, N. Ismail, M. Ismail, Toxicology study of vanillin on rats via oral and intra-peritoneal administration, Food Chem. Toxicol. 49 (2011) 25–30, https://doi.org/10.1016/j.fct.2010.08.023.
[30] E.C. Hagan, W.H. Hansen, O.G. Fitzhugh, P.M. Jenner, W.I. Jones, J.M. Taylor, E. L. Long, A.A. Nelson, J.B. Brouwer, Food flavourings and compounds of related structure. II. Subacute and chronic toxicity, Food Cosmet. Toxicol. 5 (1967) 141–157, https://doi.org/10.1016/S0015-6264(67)82961-4.
[31] W. Deichmann, K.V. Kitzmiller, On the Toxicity of Vanillin and Ethyl Vanillin for Rabbits and Rats, 1940. https://doi.org/10.1002/jps.3030291001.
[32] P.M. Jenner, E.C. Hagan, J.M. Taylor, E.L. Cook, O.G. Fitzhugh, Food flavourings and compounds of related structure I. Acute oral toxicity, Food Cosmet. Toxicol. 2 (1964) 327–343, https://doi.org/10.1016/S0015-6264(64)80192-9.
[33] K.W. Singletary, Vanilla: Potential Health Benefits, Nutr. Today 55 (2020) 186–196, https://doi.org/10.1097/NT.0000000000000412.
[34] C.F. Araújo-Lima, J. Paula da Silva Oliveira, I.L. Coscarella, C.A.F. Aiub, I. Felzenszwalb, G.P. Caprini Evaristo, A.F. Macedo, Metabolomic analysis of Cyrtopodium glutiniferum extract by UHPLC-MS/MS and in vitro antiproliferative and genotoxicity assessment, J. Ethnopharmacol. 253 (2020) 112607, https://doi.org/10.1016/j.jep.2020.112607.
[35] I.M.C.M. Rietjens, M.G. Boersma, H. Van Der Woude, S.M.F. Jeurissen, M. E. Schutte, G.M. Alink, Flavonoids and alkenylbenzenes: Mechanisms of mutagenic action and carcinogenic risk, Mutat. Res. - Fundam. Mol. Mech. Mutagen. 574 (2005) 124–138, https://doi.org/10.1016/j.mrfmmm.2005.01.028.
[36] H. Babich, A.G. Schuck, J.H. Weisburg, H.L. Zuckerbraun, Research strategies in the study of the pro-oxidant nature of polyphenol nutraceuticals, J. Toxicol. 2011 (2011), https://doi.org/10.1155/2011/467305.
[37] P.D. Gupta, T.J. Birdi, Development of botanicals to combat antibiotic resistance, J. Ayurveda Integr. Med. 8 (2017) 266–275, https://doi.org/10.1016/j.jaim.2017.05.004.
[38] E.M. Lopes, R.G. Linhares, L. de Oliveira Pires, R.N. Castro, G.H.M.F. Souza, M.G. B. Koblitz, L.C. Cameron, A.F. Macedo, Vanilla bahiana, a contribution from the Atlantic Forest biodiversity for the production of vanilla: A proteomic approach through high-definition nanoLC/MS, Food Res. Int. 120 (2019) 148–156, https://doi.org/10.1016/j.foodres.2019.02.038.
[39] D.T. Shaughnessy, R.W. Setzer, D.M. DeMarini, The antimutagenic effect of vanillin and cinnamaldehyde on spontaneous mutation in Salmonella TA104 is due to a reduction in mutations at GC but not AT sites, Mutat. Res. Mol. Mech. Mutagen 480–481 (2001) 55–69, https://doi.org/10.1016/S0027-5107(01)00169-5.
[40] A.A. King, D.T. Shaughnessy, K. Mure, J. Leszczynska, W.O. Ward, D.M. Umbach, Z. Xu, D. Ducharme, J.A. Taylor, D.M. DeMarini, C.B. Klein, Antimutagenicity of cinnamaldehyde and vanillin in human cells: Global gene expression and possible role of DNA damage and repair, Mutat. Res. - Fundam. Mol. Mech. Mutagen. 616 (2007) 60–69, https://doi.org/10.1016/j.mrfmmm.2006.11.022.
[41] G. Taner, D. Özkan Vardar, S. Aydin, Z. Aytaç, A. Başaran, N. Başaran, Use of in vitro assays to assess the potential cytotoxic, genotoxic and antigenotoxic effects of vanillic and cinnamic acid, Drug Chem. Toxicol. 40 (2017) 183–190, https://doi.org/10.1080/01480545.2016.1190740.
[42] V. Kaliappan, P. Kumaravelu, Antiproliferative effects of Vanilla planifolia leaf extract against breast cancer MCF-7 cells, Int. J. Basic Clin. Pharmacol. 8 (2018) 51, https://doi.org/10.18203/2319-2003.ijbcp20185107.
[43] K. Vijaybabu, K. Punnagai, In-vitro anti-proliferative effects of ethanolic extract of Vanilla planifolia leaf extract against A431 human epidermoid carcinoma cells, Biomed. Pharmacol. J. 12 (2019) 1141–1146, https://doi.org/10.13005/bpj/1742.
[44] K.L. Ho, L.S. Yazan, N. Ismail, M. Ismail, Apoptosis and cell cycle arrest of human colorectal cancer cell line HT-29 induced by vanillin, Cancer Epidemiol. 33 (2009) 155–160, https://doi.org/10.1016/j.canep.2009.06.003.
[45] H. Naz, M. Tarique, P. Khan, S. Luqman, S. Ahamad, A. Islam, F. Ahmad, M. I. Hassan, Evidence of vanillin binding to CAMKIV explains the anti-cancer mechanism in human hepatic carcinoma and neuroblastoma cells, Mol. Cell. Biochem. 438 (2018) 35–45, https://doi.org/10.1007/s11010-017-3111-0.
[46] P. Karande, S. Mitragotri, Enhancement of transdermal drug delivery via synergistic action of chemicals, Biochim. Biophys. Acta - Biomembr. 1788 (2009) 2362–2373, https://doi.org/10.1016/j.bbamem.2009.08.015.
[47] Y. Zhang, X. Wang, Q. Zeng, Y. Deng, P. Xie, C. Zhang, L. Huang, A new insight into synergistic effects between endogenous phenolic compounds additive and α-tocopherol for the stability of olive oil, Food Chem. 427 (2023) 136667, https://doi.org/10.1016/j.foodchem.2023.136667.