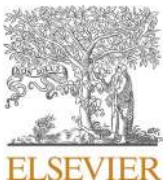
# Toxicity and therapeutic effects of aqueous extracts from reunionese medicinal plants: Insights from zebrafish models

Laura Gence, Danielle Fernezelian, Tatiana Al Anaissy, Marie Laurine Apalama, Anaïs Hoarau, Batoul Ghaddar, Matthieu Bringart, Bryan Veeren, Olivier Meilhac, Jean-Loup Bascands, et al.

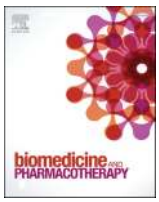
Biomedicine & Pharmacotherapy 189 (2025) 118305



Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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



# Toxicity and therapeutic effects of aqueous extracts from reunionese medicinal plants: Insights from zebrafish models

Laura Gence [a], Danielle Fernezelian [a], Tatiana Al Anaissy [a], Marie Laurine Apalama [a], Anaïs Hoarau [a], Batoul Ghaddar [a], Matthieu Bringart [a], Bryan Veeren [a], Olivier Meilhac [a,b], Jean-Loup Bascands [a,*], Nicolas Diotel [a,*]

[a] Université de La Réunion, INSERM, UMR 1188, Diabète athérothrombose Thérapies Réunion Océan Indien (DéTROI), Saint-Pierre, La Réunion, France
[b] CHU de La Réunion, Saint-Denis, La Réunion, France

ARTICLE INFO

*Keywords:*
Polyphenols
Toxicity
Tail-injury
Regeneration
Lipid accumulation

ABSTRACT

*Background:* Plant-derived compounds have long been used to treat wounds, inflammation and metabolic diseases, due to their broad biological activities making them valuable candidates for new therapies. Réunion Island has numerous medicinal plants listed in the French pharmacopoeia but limited information exists on their chemical composition and biological effects.

*Aims:* This study focused on 11 traditional medicinal plants from Réunion Island (*Antirhea borbonica, Aphloia theiformis, Ayapana triplinervis, Coffea mauritiana, Dodonaea viscosa, Hypericum lanceolatum, Nuxia verticillata, Olea europaea, Psiloxylon mauritianum, Syzygium cumini,* and *Vepris lanceolata*). We aimed to evaluate the *in vivo* toxicity of the aqueous extracts of these medicinal plants and assess their biological effects using zebrafish models.

*Methods and results:* Polyphenol concentrations and compositions were determined using the Folin-Ciocalteu colorimetric assay and liquid chromatography-mass spectrometry. The total polyphenol content ranged from 103.2 to 14.2 mg GAE/g dried plant and the main identified compounds included chlorogenic acid, gallic acid, quercetin, kaempferol, and mangiferin. Toxicity assays allowed the determination of the maximum non-toxic concentration (MNTC) for each extract. At these concentrations, several extracts showed beneficial effects on inflammation, tissue repair, and lipid accumulation.

*Conclusion:* This work presents the first comprehensive analysis of the composition and *in vivo* activity of these 11 Reunionese medicinal plants. It demonstrates the therapeutic value of such extracts in managing oxidative stress, enhancing regeneration, modulating immune responses, and reducing lipid accumulation *in vivo*. *Hypericum lanceolatum* and *Ayapana triplinervis* appeared as particularly promising. These findings support further exploration of plant-based strategies for treating chronic and metabolic diseases and their complications in a preventive/therapeutic approach.

## 1. Introduction

Traditional medicine has existed around the world for thousands of years. Evidence of human use for therapeutic purposes dates back to Neanderthal period [42]. As defined by the World Health Organization, traditional medicine refers to all the knowledge, skills and practices based on theories, beliefs and experiences of different cultures that are used to maintain health, prevent illnesses and treat diseases, especially chronic diseases [62].

In many countries, an important part of traditional medicine is herbal medicine, which includes herbs, herbal materials, herbal preparations and finished herbal products. The French Pharmacopoeia lists more than 450 examples of traditional plants for therapeutic use, 34 of which are from Réunion Island [1](Aplamedom.org). Réunion Island is a French overseas department, part of the Mascarene archipelago in the Indian Ocean, where the use of herbal medicine is widespread. In fact, the local population has used some plants for decades to treat various ailments such as fever, pain, digestive problems, hypertension, inflammation, malaria, and metabolic disorders such as diabetes and obesity [32]. In this study, 11 plants from the Reunionese biodiversity were

* Corresponding authors.
*E-mail addresses:* jean-loup.bascands@inserm.fr (J.-L. Bascands), nicolas.diotel@univ-reunion.fr (N. Diotel).

https://doi.org/10.1016/j.biopha.2025.118305
Received 8 May 2025; Received in revised form 1 July 2025; Accepted 1 July 2025
Available online 8 July 2025

selected for their potential antioxidant, anti-inflammatory, lipid-lowering and "anti-obesity/anti-diabetic" properties: *Antirhea borbonica* (*A. borbonica*), *Aphloia theiformis* (*A. theiformis*), *Ayapana triplinervis* (*A. triplinervis*), *Coffea mauritiana* (*C. mauritiana*), *Dodonaea viscosa* (*D. viscosa*), *Hypericum lanceolatum* (*H. lanceolatum*), *Nuxia verticillata* (*N. verticillata*), *Olea europaea* (*O. europaea*), *Psiloxylon mauritianum* (*P. mauritianum*), *Syzygium cumini* (*S. cumini*) and *Vepris lanceolata* (*V. lanceolata*). Traditionally, these plants are mostly prepared as beverages including infusions, decoctions and macerations [3]. These preparation methods enable the extraction of water-soluble molecules in the form of complex mixtures, among which the polyphenol family offers significant therapeutic potential. Several studies have recently identified different polyphenolic compounds in plant infusions from Reunionese vegetal biodiversity including chlorogenic acid, ferulic acid, gallic acid, mangiferin and flavonol derivatives [7,14,20,21,59]. Polyphenols are probably the most important secondary metabolites found in plants and responsible for their beneficial effects [61]. These beneficial effects have been scientifically demonstrated for some of our selected plants. For example, a crude methanolic extract of *Aphloia theiformis* leaves displayed an inhibitory effect on enzymes involved in postprandial glycemic control [45]. Different polyphenol-rich extracts from *Antirhea borbonica*, *Dodonaea viscosa* and *Syzygium cumini* were found to exhibit antioxidant and anti-inflammatory activities in hyperglycemic conditions [4,56]. However, despite traditional knowledge and studies generally performed *in vitro*, there is a lack of data about the toxicity of these medicinal plants, as well as their potential therapeutic effects *in vivo*. The challenge is consequently to better characterize their toxicity and provide evidence of their health benefits *in vivo*.

Interestingly, zebrafish (*Danio rerio*) is a well-recognized model for toxicological studies with specific standardized tests approved by the Organization for Economic Cooperation and Development (OECD) [18,40]. Notably, it shares a high degree of genomic homology with humans (more than 70 %) and the main physiological and physiopathological processes are well conserved with mammals [25]. For these reasons, zebrafish has gained considerable attention to investigate both the toxic and therapeutic effects of plant-derived compounds [30,49,55]. Zebrafish is also a relevant model for studying regeneration due to its high capacity for organ regeneration [19], and to investigate metabolic diseases such as diabetes and obesity [64].

The main objectives of this study were (1) investigating the polyphenol composition of aqueous extracts from 11 selected Reunionese medicinal plants, (2) testing their toxicity *in vivo* using zebrafish eleutheroembryo and finally (3) determining their biological properties related to inflammation, tissue regeneration and metabolic disorders. To this aim, we characterized the polyphenolic composition of the plant aqueous extracts by liquid chromatography-tandem mass spectrometry (LC-MS/MS) before assessing their toxicity in zebrafish eleutheroembryos using the OECD guidelines. Thus, a working maximum non-toxic concentration (MNTC) was determined and used for further biological and physiological investigations. Using zebrafish models, we determined the therapeutic properties of the aqueous extracts at their MNTCs, in particular on inflammatory and regenerative processes and on lipid accumulation. These data were also compared with previously published data from our laboratory on *A. borbonica*, *H. lanceolatum*, *P. mauritianum* and *A. triplinervis*.

## 2. Materials and methods

### 2.1. Plant material and preparation of the aqueous extracts

Dried leaves of the plants were purchased from the CAHEB (Coopérative Agricole des Huiles Essentielles de Bourbon, Le Tampon, Réunion Island), except for *Syzygium cumini* for which dehydrated seed powder from Habemus Papam was used. Indeed, in traditional medicine, it is the dried seeds of *Syzygium cumini* that are used. The plant names as well as their main use in traditional medicine are listed in Table 1. Note that the results presented for *A. triplinervis*, *H. lanceolatum* and *P. mauritianum* are based on our previous studies and the respective journals have provided their permissions for reuse [14,20,21].

**Table 1**
Medicinal plants used in this study and their main use in the traditional medicine of Réunion Island.

| Plant name (Industrial lot) | Vernacular name | Traditional use |
|---|---|---|
| *Ayapana triplinervis* (AYAJBGOL180710) | Ayapana | Stomach pain, inflammatory disorders, digestive problems, wounds (external use) |
| *Hypericum lanceolatum* (FJBMCA160825AA) | Fleur jaune | Gastrointestinal disorders, fever, blood detoxification |
| *Psiloxylon mauritianum* (FLBPM 20180427) | Bois de pêche marron | Amenorrhea, inflammation Diuretic and depurative properties |
| *Antirhea borbonica* (BOSRBJCA180611) | Bois d'osto | Gastric ulcers, liver inflammation, diabetes, wounds (external use) |
| *Aphloia theiformis* (CECLBRCA180411) | Change écorce | Fever, stomach pain, albuminuria, inflammation, diabetes |
| *Coffea mauritiana* (CMAJBGCA200803) | Café marron | Conjunctivitis, eye problems (external use) Diuretic properties |
| *Dodonaea viscosa* (BABMRSCA200609AA) | Bois d'arnette | Renal colic, kidney stones, osteoarthritis, gout, rheumatism |
| *Nuxia verticillata* (BMLBCA180611AA) | Bois maigre | Albuminuria, stomach ulcer Cholesterol-lowering properties |
| *Olea europaea* (BONMRSCA170821AA) | Bois d'olive noir | Infant gastroenteritis, fever, rheumatism, diabetes, diarrhea, hypertension |
| *Syzygium cumini* (JAMBLON230617) | Jamblon | Diabetes, inflammation, arthritis, blood pressure regulator |
| *Vepris lanceolata* (PPOMRSCA200706) | Patte poule | Pain, rheumatism, fever, contusions, traumas |

*Note that the traditional use described in this table was extracted from [7,32] and aplamedom.org.*

Medicinal plants are widely used in infusions. In this study, our aim was to reproduce such an aqueous extract based on the main traditional uses. To achieve this, the dried leaves were prepared in a standardized way, as previously described by our group [14,20,21]. This approach was adopted to ensure that the extract reflected traditional practices. For each medicinal plant, dried leaves were crushed using a laboratory grinder (Retsch). Aqueous extracts (infusions) were prepared by adding 50 mL of boiling E3 embryonic medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl2, 0.33 mM MgSO4) to crushed materials (10 min, under stirring, at room temperature (RT)). Each aqueous extract was filtered using a commercial filter with an estimated pore size of 20 μm, and further diluted in E3 medium to the different concentrations. Preliminary tests were conducted on each plant extract to estimate the approximate toxicity range. This started with a concentration of 20 g/L, which was obtained by infusing 2 g of dried leaves in 100 mL of boiling E3 solution (at a weight-to-volume ratio of 2 %). Then, a starting concentration was selected for each plant and a dilution series of 2 was applied in accordance with the OECD dilution guidelines.

### 2.2. Determination of total polyphenol content, flavonoid content and in vitro chemical antioxidant capacity

The Folin-Ciocalteu colorimetric assay was performed as previously described [20]. A total of 25 μL of each aqueous extract was mixed with diluted Folin–Ciocalteu reagent and sodium carbonate in a 96-well microplate, then incubated at 50 °C for 5 min. After cooling at 4 °C, the absorbance was measured at 760 nm with a UV-Visible spectrophotometer (Fluostar Omega, Bmg Labtech) and the results were expressed as mg of gallic acid equivalent (GAE)/g of dried plant. For the chemical antioxidant capacity, we used the *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) chemical assay [20]. In a 96-well microplate, 40 μL of each aqueous extract (or water as blank) was incubated with 200 μL of DPPH solution (0.25 mM in methanol) at 30 °C for 25 min.

After the colorimetric reaction, absorbance was measured at 517 nm. Results are indicated in "$IC_{50}$-like" values (in g/L) corresponding to the concentration leading to 50 % of radical scavenging activity. Each experiment was performed 3 times independently. The measurement of the flavonoid content was determined by AKINAO, a laboratory specialized in natural product chemistry and spectrometry analyses (Perpignan, France). Briefly, infusions were prepared from the different samples, using 2 g (± 0.02) of each sample that was placed in contact with 200 mL of osmosis water (pH=6.27) previously boiled (>95°C). After 10 min of stirring, infusions were filtered followed by colorimetric quantification (aluminium trichloride reagent).

### 2.3. Polyphenol identification and quantification by LC-MS/MS

Polyphenols were identified by ultra-high-performance liquid chromatography (Agilent Technologies 1260 infinity) coupled to triple quadrupole mass spectrometer (Agilent Technologies 6430) as previously described [33]. Briefly, 2 µL of the sample were injected and then separated on a Rapid Resolution Zorbax SB-C18 (2.1 ×100 mm, 1.8 µm, Agilent) at 40°C. The column was eluted with a gradient mixture of 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B) at the flow rate of 0.300 mL/min, with 1 % B at 0–3.9 min, 10 % B at 4.0–11.9 min, 20 % B at 12–12.9 min, 30 % B at 13–17.9 min, 35 % B at 18–19.9 min, 50 % B at 20–20.9, 95 % B at 21–23.9 min and 1 % B at 24–28 min. A targeted mass spectrometry approach was used to identify and quantify polyphenols. The mass spectrometer conditions were optimized as follows: spray voltage 3 kV, capillary temperature 350°C, gas flow rate 11 L/min, gas pressure 15 psi, and S lens RF level 50. The specific multiple reaction monitoring (MRM) transitions can be found on Supplementary Table 1. Data were acquired and treated with the mass hunter software (Agilent).

A calibration curve consisting of 25 purified standards was used to obtain concentration values ranging from 0.06 to 34 mg/L.

### 2.4. Zebrafish husbandry and ethics

Wild type (AB strain) and transgenic Tg(*GFAP*::GFP) zebrafish were maintained in the zebrafish facility at CYROI/DéTROI (La Réunion) under standard conditions of photoperiod (day 14 h/night 10 h) and controlled quality water (28°C, 400 µS, pH 7.4). Note that Tg(*GFAP*::GFP) was a gift from the laboratory of Dr. Sepand Rastegar (Karlsruhe Institute of Technology, EZRC), allowing the visualization of neural stem cells in the brain and the spinal cord (green fluorescence). Experiments were performed in accordance with the French and European Community Guidelines for the Use of Animals in Research (86/609/EEC and 2010/63/EU) and approved by the local Ethics Committee of CYROI and the French Government (APAFIS#2021072814123963 v6). For a better understanding, the term "eleutheroembryo" has been used in this manuscript to refer to a developmental stage from 0- to 120-hours post-fertilization (hpf).

### 2.5. Zebrafish acute toxicity tests

We performed the Fish Embryo Acute Toxicity (FET) test to study the toxic effect of the plant aqueous extracts according to the OECD guidelines [20,40]. Fertilized eggs were distributed in a 24-well plate (5 eggs/well) with 1 mL of different concentrations of the aqueous extract from: *A. borbonica* (40–2.3 g/L), *A. theiformis* (10–0.156 g/L), *C. mauritiana* (20–0.3125 g/L), *D. viscosa* (5–0.078 g/L), *N. verticillata* (20–2.5 g/L), *O. europaea* (20–0.3125 g/L), *S. cumini* (10–0.078 g/L) and *V. lanceolata* (20–0.3125 g/L). For the control condition, eggs were incubated in E3 embryonic medium. Solutions were renewed every day. The development of zebrafish eleutheroembryos was monitored from 0 to 96 h post-fertilization (hpf) to determine lethality and abnormal development by assessing (i) coagulation, (ii) somite formation, (iii) tail detachment and (iv) heartbeat. In parallel, to avoid any developmental toxicity, a similar test was performed on animals from 3- to 5-days post fertilization (dpf). Heartbeat and morphological malformations were analyzed. For each plant extract, a total of 60 animals were used per concentration tested, based on three independent experiments using 20 eleutheroembryos/concentrations. This was done for 0–4 dpf and also for 3–5 dpf. Note that the maximum non-toxic concentration (MNTC) corresponds to the highest concentration at which no death or malformations were observed.

### 2.6. Analysis of toxicity biomarkers using RT-qPCR and transgene Tg (GFAP::GFP) imaging

Eleutheroembryos were treated for 2 days (3- to 5-dpf) with E3 and the aqueous extracts at the MNTCs (Table 5**, Result part**). Then, eleutheroembryos were processed for RT-qPCR analyses for key genes recognized as biomarkers of hepatoxicity (*fabp10a*), cardiotoxicity (*erg*), nephrotoxicity (*ctgf*) and glucocorticoid responsive-gene (stress) (*fkbp5*). RNA extraction, cDNA synthesis and qPCR were performed as previously described [20]. Specific zebrafish primers are provided in Table 2. Three independent experiments were done on a total of 150 animals/condition (3 pools of 50 eleutheroembryos).

For transgene expression analysis, Tg(*GFAP::*GFP) eleutheroembryos allowing GFP expression in neural stem cells (GFAP-positive cells) were treated for 2 days (3- to 5-dpf) with E3 and the aqueous extracts at the MNTCs (Table 5**, Result part**). Then, the potential toxic effect on the nervous system was evaluated by measuring the GFP-fluorescence intensity as previously described [20]. Three independent experiments were carried out on a total of 30 eleutheroembryos/condition.

### 2.7. Study of immune cell recruitment and tissue regeneration in a model of tail amputation

Experiments were carried out on 3-dpf WT eleutheroembryos according to our previous study [20]. Briefly, 3-dpf eleutheroembryos were anesthetized (0.02 % tricaine) and transections were performed close to the notochord under a stereomicroscope using a scalpel. After the amputation, eleutheroembryos were incubated in E3 and the plant aqueous extracts at their respective MNTC for 6 h (immune cell recruitment analysis) or 3 days (regeneration analysis). For immune cell recruitment, eleutheroembryos were fixed in 4 % paraformaldehyde (PFA) and processed for immunohistochemistry analyses (see below). For tail regeneration, images were taken under anesthesia at 0- and 3-day post-lesion and the regenerated area was quantified using ImageJ software. These experiments were performed 3 times independently on a

**Table 2**
Zebrafish primer sequences.

| Zebrafish gene | Gene name | Biomarker of | Primer sequences |
|---|---|---|---|
| *fapb10a* | *fatty acid binding protein 10a* | Hepatotoxicity | F: CCAGTGACAGAAATCCAGCA R: GTTCTGCAGACCAGCTTTCC |
| *erg* | *ether-a-go-go related gene* | Cardiotoxicity | F: CAGATGCTCCGTGTGAAAGA R: TGCGGTTCAGATGAAGACAG |
| *ctgf* | *connective tissue growth factor* | Nephrotoxicity | F: CTCCCCAAGTAACCGTCGTA R: TCCACCAAACACACAAGTGG |
| *fkbp5* | *FK506 binding protein 51* | Glucocorticoid signaling | F: CAAAAGGGGGAATGCTGTT R: TTCTTTTCTGCCCTCTTTGC |
| *ef1α* | *elongation factor 1-alpha* | Housekeeping gene | F: AGCAGCAGCTGAGGAGTGAT R: CCGCATTTGTAGATCAGATGG |

total of 30–45 eleutheroembryos/condition.

### 2.8. Immunohistofluorescence

Myeloperoxidase (Mpo) and L-Plastin (Lcp1) whole-mount immunostainings were performed to label neutrophils and macrophages, respectively [14,20], using rabbit anti-Mpo (1:200, Abcam, ab210563), anti-Lcp1 (1:200, GeneTex, GTX124420), Alexa Fluor® 488 and 594 donkey anti-rabbit (1:500, Abcam, ab150073, ab150076) antibodies, with DAPI counterstaining (1 ng/mL, ThermoFisher, D1306).

### 2.9. High-fat diet (HFD) zebrafish model and Oil Red O (ORO) staining

A HFD model was established by overfeeding 7-dpf old larvae [20]. Briefly, 7-dpf larvae were divided in 3 different conditions: normal diet (Control), overfed (HFD) and HFD supplemented with the plant aqueous extracts at the MNTCs. Control larvae were incubated with 0.1 % dry food (Gemma Micro 75, Skretting) whereas overfed larvae were fed with 0.1 % egg yolk (Sigma) for 5 days. The solutions were renewed daily from 7- to 11-dpf. At the end of the experiment, larvae were fixed in 4 % PFA and processed for the Oil red O (ORO) staining (n = 45 larvae/-condition from 3 independent experiments).

Fixed larvae were washed with 1X PBS/0.1 % Tween-20 (PTw), dehydrated in methanol and then stained overnight at RT with 0.15 % ORO (Sigma) (in 60 % of isopropanol/1X PBS). Next day, larvae were rehydrated in PTw for imaging. Relative quantification of ORO staining was carried out by measuring the absorbance at 500 nm for each larva after overnight incubation in 200 μL of 60 % isopropanol, under stirring.

### 2.10. Microscopy

Pictures were acquired using a Nikon eclipse 80i equipped with a Hamamatsu camera, a Nikon SMZ18 stereomicroscope equipped with a Nikon DS-Fi3 Camera and the respective NIS elements software. Images were analyzed using the ImageJ software (https:// imagej.nih.gov/ij/) by two different users blind to the treatment.

### 2.11. Statistical analysis

Data are expressed as the mean ± SD (Standard Deviation). Statistical analyses were performed with GraphPad Prism 9 software. Comparisons between groups were determined using Student *t*-test or one-way ANOVA for multiple comparisons. *p*-values $< 0.05$ were considered statistically significant.

## 3. Results

### 3.1. Polyphenol content and composition and antioxidant capacity

In Réunion Island, aqueous extracts of many plants are commonly used in traditional medicine for counteracting a multitude of disorders. These extracts are rich sources of bioactive molecules including polyphenols but their phytochemical composition remains to be determined. We first assessed the total polyphenol content through the Folin-Ciocalteu method (Table 3). As shown, the aqueous extracts from *H. lanceolatum* (103.2 ± 6.1 mg GAE/g of dried plant), *S. cumini* (86.0 ± 11.9) and *P. mauritianum* (57.9 ± 7.2) were the richer in polyphenolic compounds. The 8 other plant extracts were found with significant lower total polyphenol contents ($p < 0.001$) ranging from 14.2 ± 1.1–28.9 ± 6.0 mg GAE/g of dried plant. We also reported high flavonoid levels in *H. lanceolatum* and *A. theiformis* aqueous extracts (29.0 ± 6.0 and 26.0 ± 5.0 mg QE/g, respectively). Regarding the other extracts, the flavonoid contents ranged between 2.0 and 6.0 mg QE/g dried plant.

Given that the antioxidant properties of plants are often related to their polyphenol content, we next evaluated the chemical antioxidant activity of the plant aqueous extracts using the DPPH assay using ascorbic acid as a positive control (Table 3). As expected, the plant richest in polyphenols (*H. lanceolatum*, *S. cumini* and *P. mauritianum*) showed $IC_{50}$-like values lower than the others (0.5–1.1 *versus* 2.1–23.1 g/L), testifying their stronger chemical antioxidant capacity.

In parallel, the identification of polyphenolic molecules was performed by high-resolution mass spectrometry (LC-MS/MS). The analysis allowed the identification of major polyphenolic compounds in the aqueous extracts (Table 4 and Supplementary Figure 1) including chlorogenic acid (almost present in all the extracts except in *A. theiformis*, *S. cumini* and *V. lanceolata* extracts where it was not detected), gallic acid (abundant in *P. mauritianum* and *S. cumini* extracts) and ferulic acid isomers (*A. triplinervis* extract). Flavanols (procyanidin and epicatechin) were mostly detected in the aqueous extract of *D. viscosa* (44 % of the total compounds identified). Many flavonols such as quercetin, kaempferol and derivatives were also present in the extracts, particularly in the *H. lanceolatum*, *P. mauritianum* and *O. europaea* aqueous extracts. Of interest, mangiferin isomers were mainly quantified in *A. theiformis* extract, representing almost 99 % of total compounds identified.

### 3.2. In vivo acute toxicity in zebrafish eleutheroembryos

Performing the OECD test (Fish Embryo Acute Toxicity (FET)), we monitored the acute toxicity of our plant aqueous extracts in zebrafish eleutheroembryos from 0- to 96-hpf. As previously described [20], we also used 3- to 5-dpf animals in order to exclude any embryonic toxicity knowing that at these developmental stages, zebrafish

**Table 3**
Total polyphenol (in gallic acid equivalent, GAE) and flavonoid (in quercetin equivalent, QE) contents, and chemical antioxidant capacity of the plant aqueous extracts (results are expressed as means ± SD of three independent experiments).

| | **Total polyphenol content** (mg GAE/g dried plant) | **Flavonoid content** (mg QE/g dried plant) | **Antioxidant capacity** $IC_{50}$-like values (g/L) |
|---|---|---|---|
| *Ayapana triplinervis*(1) | 24.9 ± 1.9 | 3.0 ± 1.0 | 5.4 ± 1.1 |
| *Hypericum lanceolatum*(2) | 103.2 ± 6.1 | 29.0 ± 6.0 | 0.9 ± 0.1 |
| *Psiloxylon mauritianum*(3) | 57.9 ± 7.2 | 5.0 ± 1.0 | 1.1 ± 0.2 |
| *Antirhea borbonica* | 15.1 ± 1.1 | 5.0 ± 1.0 | 4.5 ± 0.3 |
| *Aphloia theiformis* | 21.8 ± 3.2 | 26.0 ± 5.0 | 2.1 ± 0.4 |
| *Coffea mauritiana* | 14.2 ± 5.1 | 2.0 ± 1.0 | 10.3 ± 0.9 |
| *Dodonaea viscosa* | 28.9 ± 6.0 | 4.0 ± 1.0 | 2.2 ± 0.2 |
| *Nuxia verticillata* | 18.7 ± 3.5 | 4.0 ± 1.0 | 5.8 ± 0.5 |
| *Olea europaea* | 18.5 ± 1.7 | 6.0 ± 1.0 | 4.6 ± 0.2 |
| *Syzygium cumini* | 86.0 ± 11.9 | 3.0 ± 1.0 | 0.5 ± 0.1 |
| *Vepris lanceolata* | 16.6 ± 1.7 | 2.0 ± 1.0 | 23.1 ± 8.7 |
| **Ascorbic acid** | - | - | 0.08 ± 0.01 |

*Note that for the 3 first plant extracts, the data presented comes from our previous studies (*(1) Fernezelian et al. [14], (2) Gence et al. [20], (3) Ghaddar et al. [21]*).*

**Table 4**
Polyphenol identification and quantification in the plant aqueous extracts by LC-MS/MS.

| Plant aqueous extract | Compound identified | Content (μg/mL) |
|---|---|---|
| *A. triplinervis* | Caffeic acid | 0.08 |
| | Ferulic acid | 0.23 |
| | **Ferulic acid isomers** | **13.70 (72.2 %)** |
| | Isoferulic acid | 0.05 |
| | Caffeoyl glucarate isomers | 0.30 |
| | **5-Caffeoylquinic acid (Chlorogenic acid)** | **2.38 (12.5 %)** |
| | Caffeoylquinic acid isomers | 0.40 |
| | Neochlorogenic acid | 0.03 |
| | Cryptochlorogenic acid | 0.11 |
| | Cynarin isomers | 0.10 |
| | Kaempferol acetyl-glucoside isomers | 0.10 |
| | Kaempferol hexoside isomers | 0.10 |
| | Quercetin 3-glucoside | 0.06 |
| | **Quercetin 3-galactoside** | **0.84 (4.4 %)** |
| | Quercetin acetyl-hexoside isomers | 0.20 |
| | Mangiferin isomers | 0.30 |
| *H. lanceolatum* | Gallic acid | 0.85 |
| | Caffeic acid | 0.80 |
| | Ferulic acid | 0.06 |
| | Feruoylquinic acid isomers | 3.60 |
| | Caffeoyl glucarate isomers | 2.20 |
| | **5-Caffeoylquinic acid (Chlorogenic acid)** | **799.91 (66.5 %)** |
| | Caffeoylquinic acid isomers | 3.50 |
| | **Neochlorogenic acid** | **72.78 (6.1 %)** |
| | Cryptochlorogenic acid | 7.55 |
| | Cynarin isomers | 14.00 |
| | Epicatechin | 1.07 |
| | Procyanidin type B | 1.65 |
| | Procyanidin C1 | 1.02 |
| | Procyanidin A2 | 0.06 |
| | Kaempferol | 1.32 |
| | Kaempferol 3-glucoside | 0.15 |
| | Kaempferol acetyl-glucoside isomers | 1.10 |
| | Kaempferol hexoside isomers | 1.10 |
| | Kaempferol pentoside isomers | 0.20 |
| | Kaempferol 3-rutinoside | 1.55 |
| | Kaempferol rutinoside isomers | 2.60 |
| | **Quercetin** | **46.30 (3.8 %)** |
| | **Quercetin 3-glucoside** | **54.68 (4.5 %)** |
| | **Quercetin 3-galactoside** | **97.22 (8.1 %)** |
| | Quercetin acetyl-hexoside isomers | 1.80 |
| | Quercetin hexoside isomers | 1.60 |
| | Quercetin pentoside isomers | 0.10 |
| | **Quercetin 3-rhamnoside** | **34.93 (2.9 %)** |
| | **Quercetin 3-rutinoside** | **46.55 (3.9 %)** |
| | Taxifolin (dihydroquercetin) | 0.03 |
| | Mangiferin isomers | 2.60 |
| *P. mauritianum* | **Gallic acid** | **6.42 (18.3 %)** |
| | 5-Caffeoylquinic acid (Chlorogenic acid) | 0.60 |
| | Caffeoylquinic acid isomers | 3.60 |
| | Catechin | 0.07 |
| | Procyanidin type B | 0.19 |
| | Kaempferol | 0.41 |
| | **Kaempferol 3-glucoside** | **14.53 (41.4 %)** |
| | Kaempferol acetyl-glucoside isomers | 0.20 |
| | **Kaempferol acetyl-rhamnoside isomers** | **4.80 (13.7 %)** |
| | Kaempferol acetyl-pentoside isomers | 0.70 |
| | Kaempferol hexoside isomers | 0.50 |
| | Kaempferol pentoside isomers | 0.70 |
| | Kaempferol glucuronide isomers | 0.40 |
| | Quercetin | 0.23 |
| | Quercetin 3-glucoside | 1.42 |
| | Quercetin 3-galactoside | 0.18 |
| | Quercetin 3-rhamnoside | 0.13 |
| *A. borbonica* | Gallic acid | 0.04 |
| | Caffeic acid | 0.28 |
| | Ferulic acid | 0.02 |
| | **5-Caffeoylquinic acid (Chlorogenic acid)** | **3.11 (28.7 %)** |
| | **Caffeoylquinic acid isomers** | **1.10 (10.1 %)** |
| | Neochlorogenic acid | 0.51 |
| | Cryptochlorogenic acid | 0.97 |
| | Cynarin isomers | 0.70 |
| | Kaempferol hexoside isomers | 0.10 |
| | Kaempferol 3-rutinoside | 0.18 |
| | Kaempferol rutinoside isomers | 0.50 |
| | Quercetin 3-glucoside | 0.43 |
| | Quercetin 3-galactoside | 0.30 |
| | **Quercetin 3-rutinoside** | **2.60 (24.0 %)** |
| *A. theiformis* | Gallic acid | 0.08 |
| | Epigallocatechin | 0.05 |
| | Procyanidin type B | 0.08 |
| | Procyanidin C1 | 0.12 |
| | Procyanidin A2 | 0.33 |
| | Quercetin 3-glucoside | 0.06 |
| | Quercetin hexoside isomers | 0.10 |
| | **Mangiferin isomers** | **56.9 (98.6 %)** |
| *C. mauritiana* | Gallic acid | 0.05 |
| | Caffeic acid | 0.08 |
| | Ferulic acid | 0.05 |
| | Isoferulic acid | 0.04 |
| | Caffeoyl glucarate isomers | 0.20 |
| | **5-Caffeoylquinic acid (Chlorogenic acid)** | **19.75 (63.6 %)** |
| | Caffeoylquinic acid isomers | 0.60 |
| | **Neochlorogenic acid** | **4.19 (13.5 %)** |
| | **Cryptochlorogenic acid** | **2.90 (9.3 %)** |
| | Cynarin isomers | 0.10 |
| | Kaempferol 3-glucoside | 0.04 |
| | Kaempferol acetyl-glucoside isomers | 0.10 |
| | Kaempferol 3-rutinoside | 0.69 |
| | Kaempferol rutinoside isomers | 1.30 |
| | Quercetin 3-glucoside | 0.01 |
| | Quercetin 3-rutinoside | 0.86 |
| | Mangiferin isomers | 0.10 |
| *D. viscosa* | Gallic acid | 0.59 |
| | Caffeic acid | 0.15 |
| | Ferulic acid | 0.08 |
| | Caffeoyl glucarate isomers | 1.30 |
| | 5-Caffeoylquinic acid (Chlorogenic acid) | 0.35 |
| | Caffeoylquinic acid isomers | 0.40 |
| | Neochlorogenic acid | 1.76 |
| | **Cryptochlorogenic acid** | **3.18 (10.3 %)** |
| | Catechin | 0.04 |
| | Epicatechin | 2.60 |
| | Epigallocatechin | 0.03 |
| | **Procyanidin type B** | **5.88 (19.0 %)** |
| | **Procyanidin C1** | **3.30 (10.7 %)** |
| | Procyanidin A2 | 0.94 |
| | Procyanidin tetramer | 0.71 |
| | Kaempferol 3-glucoside | 0.05 |
| | Kaempferol hexoside isomers | 0.30 |
| | Kaempferol glucuronide isomers | 0.20 |
| | **Kaempferol 3-rutinoside** | **2.82 (9.1 %)** |
| | **Kaempferol rutinoside isomers** | **4.70 (15.2 %)** |
| | Quercetin 3-glucoside | 0.05 |
| | Quercetin 3-galactoside | 0.03 |
| | Quercetin 3-glucuronide | 0.65 |
| | Quercetin 3-rutinoside | 0.86 |
| *N. verticillata* | Gallic acid | 0.15 |
| | Caffeic acid | 0.12 |
| | Ferulic acid | 0.07 |
| | 5-Caffeoylquinic acid (Chlorogenic acid) | 0.04 |
| | **Kaempferol hexoside isomers** | **1.30 (8.4 %)** |
| | **Kaempferol glucuronide isomers** | **12.60 (81.7 %)** |
| | Kaempferol rutinoside isomers | 0.40 |
| | Quercetin 3-glucoside | 0.03 |
| | Quercetin hexoside isomers | 0.50 |
| | Quercetin 3-rutinoside | 0.21 |
| *O. europaea* | Gallic acid | 0.04 |

(*continued on next page*)

**Table 4** (*continued*)

| Plant aqueous extract | Compound identified | Content (µg/mL) |
|---|---|---|
| | Caffeic acid | 0.11 |
| | Ferulic acid | 0.21 |
| | Isoferulic acid | 0.08 |
| | 5-Caffeoylquinic acid (Chlorogenic acid) | 1.09 |
| | Caffeoylquinic acid isomers | 0.40 |
| | Cryptochlorogenic acid | 0.04 |
| | Kaempferol | 0.03 |
| | **Kaempferol hexoside isomers** | **27.3 (40.5 %)** |
| | Kaempferol glucuronide isomers | 0.10 |
| | Kaempferol 3-rutinoside | 0.05 |
| | Kaempferol rutinoside isomers | 1.70 |
| | **Quercetin** | **26.11 (38.7 %)** |
| | Quercetin 3-glucoside | 0.49 |
| | Quercetin 3-galactoside | 0.96kim |
| | **Quercetin hexoside isomers** | **7.80 (11.6 %)** |
| | Quercetin 3-rhamnoside | 0.04 |
| | Quercetin 3-rutinoside | 0.70 |
| | Taxifolin (dihydroquercetin) | 0.17 |
| *S. cumini* | **Gallic acid** | **13.92 (99.5 %)** |
| | Gallocatechin | 0.07 |
| *V. lanceolata* | Gallic acid | 0.10 |
| | Caffeic acid | 0.05 |
| | **Ferulic acid** | **0.24 (13.3 %)** |
| | Isoferulic acid | 0.10 |
| | **Quercetin 3-glucoside** | **0.33 (18.3 %)** |
| | **Quercetin 3-rutinoside** | **0.98 (54.4 %)** |

For greater clarity, polyphenolic compounds have been grouped by family: **phenolic acids** (gallic, caffeic, ferulic and chlorogenic acids, cynarin and their respective derivatives), **flavanols** (catechin, epicatechin, procyanidins and derivatives), **flavonols** (kaempferol, quercetin and derivatives), **dihydroflavonols** (taxifolin) and **xanthones** (mangiferin and derivatives). Note that compounds in bold correspond to the major polyphenols identified for each extract.

eleutheroembryos can metabolize, detoxify or excrete some toxins and other exogenous substances due to functional liver and kidney [12,15]. Briefly, newly fertilized embryos were incubated with different concentrations of the different aqueous extracts from 0 to 96-hpf and every 24 h, the eggs were carefully checked for coagulation, somite formation, tail detachment and heartbeat as indicators of abnormal development and lethality. Similarly, heartbeat and morphological malformations (spinal curvature and pericardial edema) were analyzed for zebrafish eleutheroembryos from 3- to 5-dpf. The FET test allowed us to determine the median lethal concentration ($LC_{50}$) which corresponds to the concentration leading to 50 % of lethality, for each plant extract (Table 5). For 6 plants, the $LC_{50}$ determined from 3 to 5-dpf was significantly higher than from 0 to 96 hpf. Of these 6 plants, a higher concentration was needed to reach the $LC_{50}$ for 4 of them, suggesting that at a later stage, zebrafish eleutheroembryos may have a better detoxification capacity.

Finally, the study of these morphological markers enables the determination of the maximum non-toxic concentration (MNTC) for each infusion where no mortality and developmental alterations were observed. It should be noted that these MNTCs were determined from the model using 3- to 5-dpf eleutheroembryos since the following experiments used animals at this developmental stage. In addition, at this stage, they have functional detoxification organs including liver and kidneys [12,15].

To further ascertain the non-toxic effects of the plant extracts, we analyzed the gene expression of biomarkers of hepatotoxicity (*fabp10a*), cardiotoxicity (*erg*), nephrotoxicity (*ctgf*) and stress (*fkbp5*, a target gene of glucocorticoid signaling) after the treatment of 3-dpf eleutheroembryos with the aqueous extracts for 2 days. In general, our results showed no significant change in the expression of these genes for all plant aqueous extracts (Fig. 1). However, for some plant extracts including *A. theiformis* and *V. lanceolata* extracts, the expression of *ctgf*, *fkbp5* and *fapb10a* genes were barely but significantly decreased in the treated eleutheroembryos in comparison to the controls. The expression of other genes involved in liver and renal homeostasis such as *gclc* (glutamate cysteine ligase, catalytic subunit) and *kim1* (kidney injury molecule 1) was also analyzed and no difference was observed in the expression of these genes after both treatments with *A. theiformis* and *V. lanceolata* extracts (data not shown), reinforcing the fact that these concentrations have no obvious toxic effects.

**Table 5**
*In vivo* acute toxicity assays in zebrafish eleutheroembryos at 2 different developmental stages. Estimated median lethal ($LC_{50}$) and maximum non-toxic (MNTC) concentrations determined from 3- to 5-dpf exposure (results are expressed as means ± SD of three independent experiments).

| | $LC_{50}$ (g/L) | | MNTC (g/L) | Estimated polyphenol content in MNTC |
|---|---|---|---|---|
| | 0- to 96-hpf | 3- to 5-dpf | | |
| *A. triplinervis*(1) | 3.5 ± 0.2 | 2.7 ± 0.0 | 1.25 | 31.1 mg |
| *H. lanceolatum*(2) | 1.6 ± 0.5 | 1.0 ± 0.2 | 0.3125 | 32.2 mg |
| *P. mauritianum*(3) | 0.7 ± 0.0 | 0.8 ± 0.1 | 0.556 | 32.2 mg |
| *A. borbonica*(4) | 17.5 ± 0.5 | 20.1 ± 0.0* | 8.4375 | 127.4 mg |
| *A. theiformis* | 4.8 ± 0.9 | 2.3 ± 0.5* | 0.625 | 13.6 mg |
| *C. mauritiana* | 7.2 ± 1.7 | 11.2 ± 1.3* | 3.75 | 53.3 mg |
| *D. viscosa* | 0.4 ± 0.0 | 0.5 ± 0.2 | 0.3125 | 9.0 mg |
| *N. verticillata* | 13.2 ± 2.2 | 12.1 ± 0.2 | 2.5 | 46.8 mg |
| *O. europaea* | 5.6 ± 0.8 | 15.4 ± 0.0* | 10 | 185 mg |
| *S. cumini* | 2.1 ± 0.7 | 0.9 ± 0.0* | 0.156 | 13.4 mg |
| *V. lanceolata* | 3.0 ± 0.9 | 6.1 ± 0.9* | 1.25 | 20.8 mg |

hpf, hour post-fertilization. dpf, day post-fertilization. The * indicates significant differences between 0- to 96-hpf and 3- to 5-dpf groups (Student *t*-test, $p < 0.05$). Note that for the 4 first plant extracts, the data presented comes from our previous studies ((1) Fernezelian et al. [14], (2) Gence et al. [20], (3) Ghaddar et al. [21], (4) Veeren et al. [59]).

The potential deleterious impact of the plant extracts on the central nervous system was determined using Tg(*GFAP::GFP*) eleutheroembryos, in which GFP protein is expressed in neural stem cells (NSC) reflecting their reactivity through GFP expression. Interestingly, the treatment of the eleutheroembryos for 2 days (from 3- to 5-dpf) with *A. borbonica*, *A. theiformis* and *S. cumini* aqueous extracts resulted in a small but significant increase in GFP expression. In contrast, *C. mauritiana*, *D. viscosa*, *N. verticillata* and *O. europaea* led to a low but significant decrease in GFP expression. *A. triplinervis*, *H. lanceolatum*, *P. mauritianum* and *V. lanceolata* had no effect in GFP fluorescence intensity in the spinal cord (Fig. 2). However, these changes remained relatively low (< 20 %), raising the question of a real physiological impact of these plant extracts on the nervous system.

Overall, at their respective MNTCs, the plant aqueous extracts did not exhibit toxic effects on the liver, heart, kidney and central nervous system in zebrafish at these developmental stages. We consequently used these concentrations (Table 2) to investigate the biological properties of the plant extracts in the rest of our study.

### 3.3. Aqueous extracts of medicinal plants modulate the inflammatory and regenerative processes in a model of caudal tail amputation

Given the use of these plants in traditional medicine mainly for their anti-inflammatory properties, we decided to study the immunomodulatory effects of the extracts by monitoring the recruitment of immune cells in a tail injury model performed in zebrafish as previously described [20]. It is known that 6 h post-amputation Mpo-positive (neutrophils) and Lcp1-positive (macrophages) are recruited at the injured site. In the present work, we show that the treatment of zebrafish eleutheroembryos for 6 h post-amputation with *A. triplinervis*, *H. lanceolatum*, *P. mauritianum*, *A. borbonica*, *D. viscosa*, *O. europaea* and *V. lanceolata* extracts resulted in the increase in $Mpo^+$ and/or $Lcp1^+$ cells to the injured site compared to controls (Figs. 3 and 4). On the contrary, other medicinal plant extracts including those of *A. theiformis*,

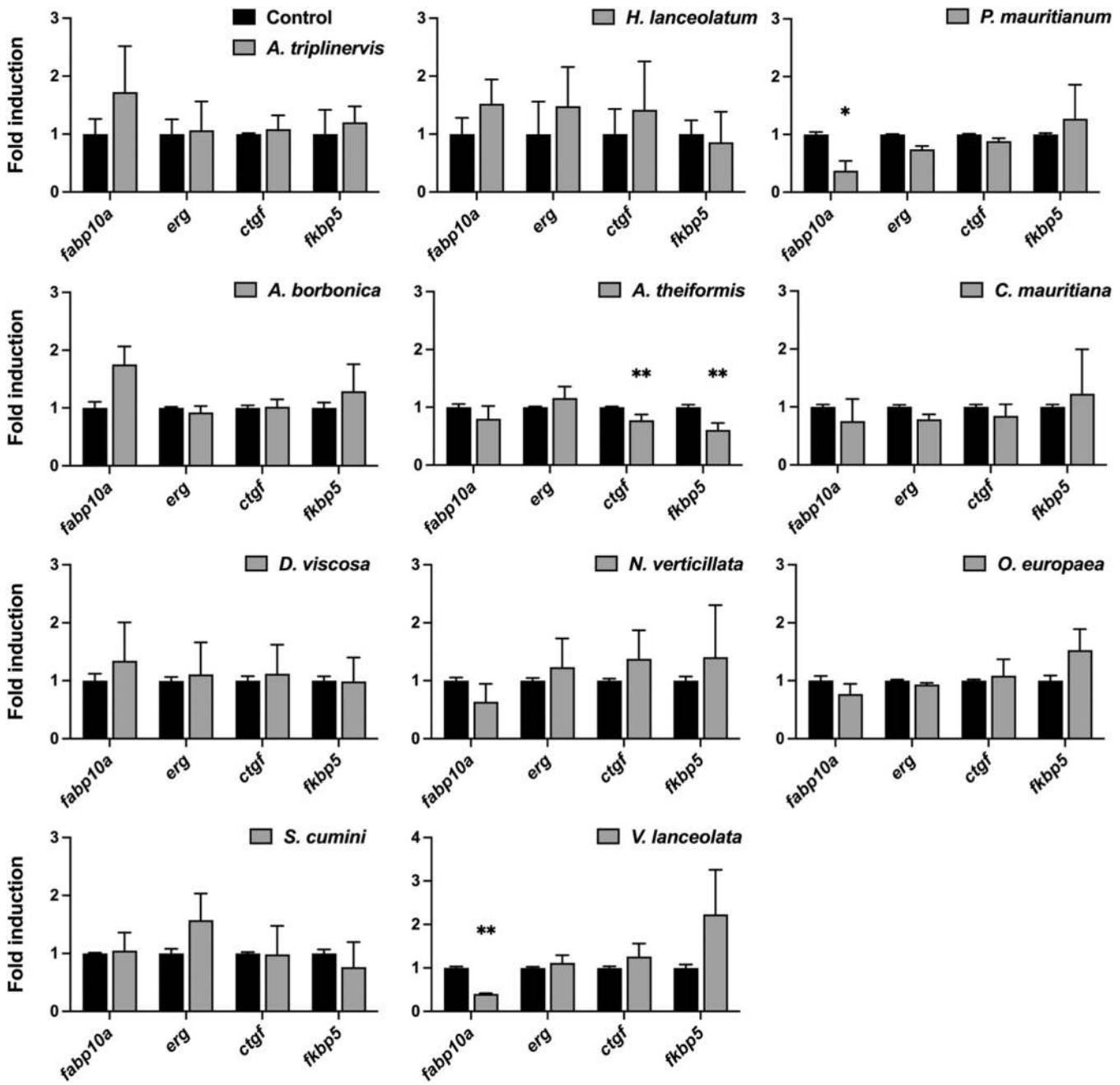



**Fig. 1. Effects of 11 medicinal plant extracts on expression of toxicity genes.** qPCR analysis of recognized biomarkers for hepatotoxicity (*fabp10a*), cardiotoxicity (*erg*), nephrotoxicity (*ctgf*) and glucocorticoid signaling (*fkpb5*) of eleutheroembryos treated with 11 aqueous extracts at their respective MNTCs and E3 medium (Control) for 2 days. Results are expressed as means ± SD (n = 6 pools of 20 eleutheroembryos/group from 3 independent experiments). *p < 0.05, **p < 0.01 (Student *t*-test).

*C. mauritiana*, *N. verticillata* and *S. cumini* had no significant effect on the recruitment of immune cells in these experimental conditions compared to control animals. Interestingly, except for *A. borbonica* and *O. europaea* aqueous extracts, the increase in neutrophils ($Mpo^+$ cells) to the injury site was associated with the increase in macrophages ($Lcp1^+$ cells).

In a next step, knowing that immune cells are essential throughout tissue repair, we took advantage of the high ability of zebrafish to regenerate and use the same tail amputation model to assess the regeneration after the treatment with the plant extracts for 3 days. As shown in Fig. 5, tail regeneration of treated eleutheroembryos with *A. triplinervis* and *H. lanceolatum* was significantly improved compared to untreated animals. Surprisingly, most of the other aqueous extracts (*A. borbonica*, *A. theiformis*, *C. mauritiana*, *D. viscosa*, *O. europaea* and *S. cumini* extracts) limited tail regeneration in treated eleutheroembryos compared to controls while treatment with *P. mauritianum*, *N. verticillata* and *V. lanceolata* did not alter tail regeneration.

Together, these data showed that the increased recruitment of immune cells at the damaged site by some plant extract during tissue repair was not necessarily associated with pro-regenerative activities. Indeed, these results highlighted the potential impact of the aqueous extracts on other processes contributing to the tissue regeneration.

### *3.4. Aqueous extracts of medicinal plants exhibited beneficial effects in a HFD model*

Lastly, we investigated the potential effects of these 11 medicinal

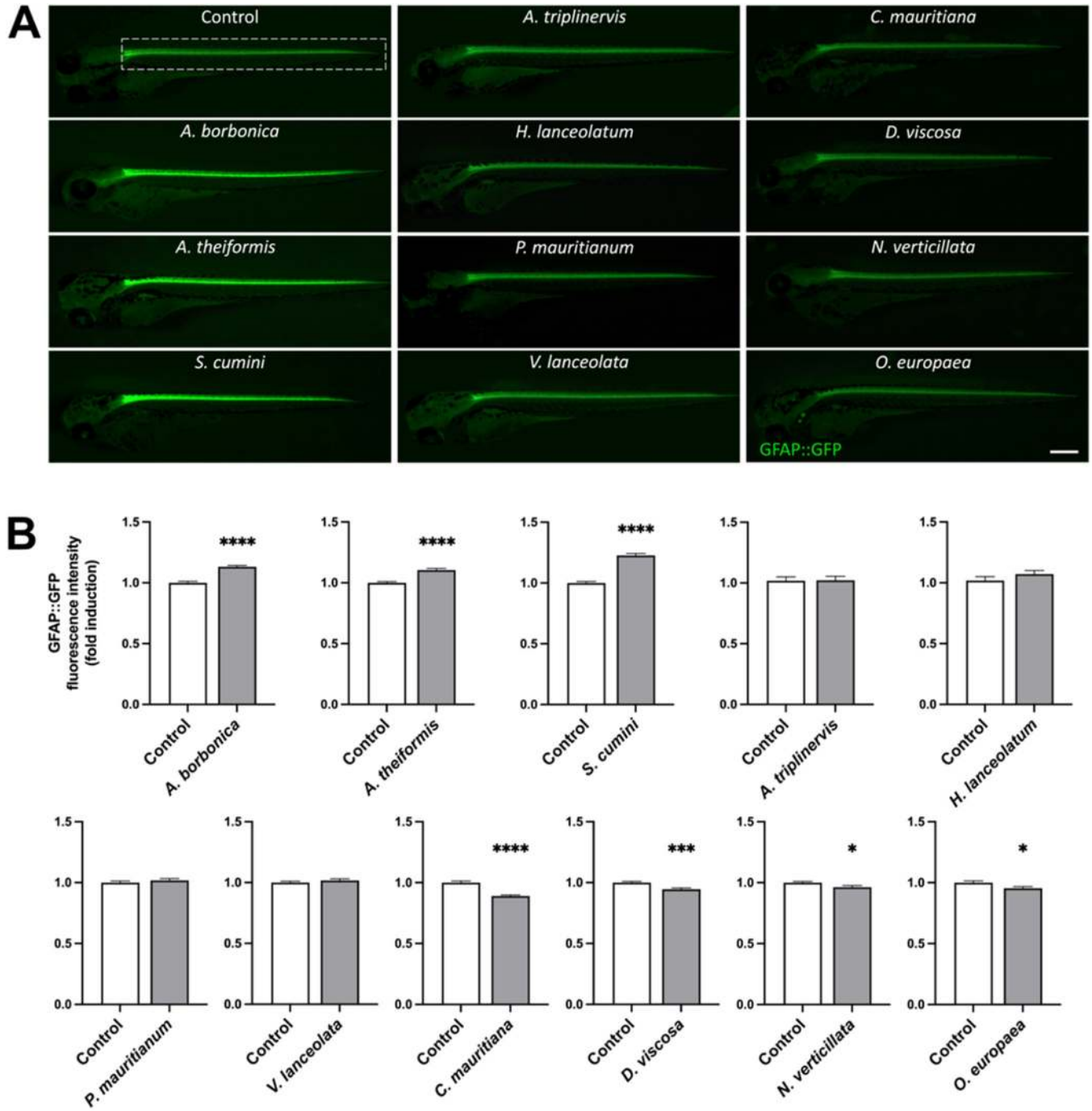



**Fig. 2. Effects of 11 medicinal plant extracts on nervous system homeostasis using *Tg(GFAP::GFP)* zebrafish eleutheroembryos. (A)** Representative pictures of *Tg(GFAP::GFP)* eleutheroembryos treated with E3 medium (Control) and 11 plant aqueous extracts at the respective MNTCs for 48 h. **(B)** Quantification of the GFAP::GFP fluorescence in the spinal cord (dotted rectangle) at the end of the experiment. Results are expressed as means ± SD (n = 35–59/group from 3 independent experiments). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 (Student *t*-test). Bar = 300 μm.

plant extracts given their traditional uses for the management of metabolic disorders. Indeed, many plants and in particular their bioactive molecules including polyphenols may act on lipid metabolism [6]. For that, we performed a HFD model in zebrafish larvae and monitored the lipid-lowering effect of our plant extracts at the MNTC. We showed that overfed larvae with 0.1 % egg yolk powder (HFD) exhibited higher Oil Red O (ORO) staining of neutral lipids (such as triglycerides and cholesterol esters) than controls (normally fed), reflecting an increased accumulation of lipids (Fig. 6). Except for *A. theiformis* and *V. lanceolata* extracts, overfed larvae treated with *A. borbonica*, *A. triplinervis*, *C. mauritiana*, *D. viscosa*, *H. lanceolatum*, *N. verticillata*, *O. europaea*, *P. mauritianum* and *S. cumini* extracts displayed a significant lower ORO staining compared to untreated HFD larvae. Through this simple method, our results suggested the interesting lipid-lowering properties of most of our plant aqueous extracts at their MNTCs.

## 4. Discussion

Among the medicinal plants found in Réunion Island, some plants such as *A. borbonica*, *A. theiformis*, *A. triplinervis*, C. *mauritiana*, *D. viscosa*, *H. lanceolatum*, *N. verticillata*, *O. europaea*, *P. mauritianum*, *S. cumini* and *V. lanceolata* are widely used in traditional medicine for their

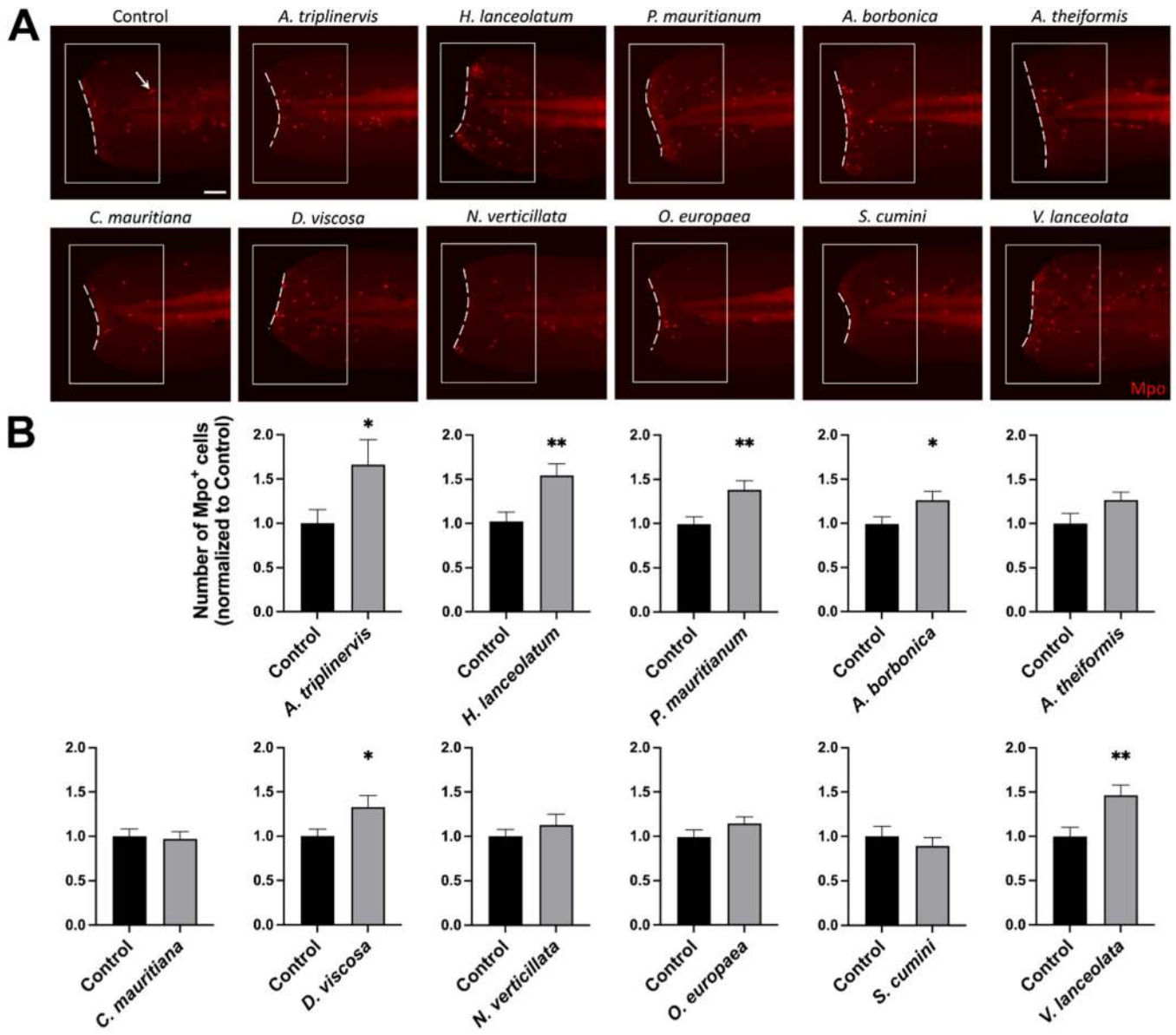



**Fig. 3. Effects of 11 medicinal plant extracts on recruitment of neutrophils after tail injury. (A)** Representative pictures of tail amputations of 3-dpf eleutheroembryos, incubated with E3 medium (Control) and the plant aqueous extract at the respective MNTCs for 6 h followed by Mpo-immunostaining. **(B)** Normalized number of Mpo-positive (neutrophils) cells (arrows) at the injury site (white box). Dotted lines represent the tail amputation site. Results are expressed as means ± SD (n = 30/group from 3 independent experiments). *p < 0.05, **p < 0.01 (Student *t*-test). Bar = 60 µm.

antioxidant and anti-inflammatory activities as well as for their reputed effectiveness in the treatment of metabolic disorders, including diabetes and obesity[32]. However, they are poorly characterized for their potential toxicity and their real therapeutic effects *in vivo* are largely unknown. In this study, we investigated for the first time the toxicity of aqueous extracts from 11 selected plants in zebrafish according to the OECD guidelines and determined their respective MNTCs. At these respective maximum non-toxic concentrations, we showed that some aqueous extracts (*A. borbonica*, *A. triplinervis*, *D. viscosa*, *H. lanceolatum*, *O. europaea*, *P. mauritianum* and *V. lanceolata* extracts) increased the recruitment of immune cells after tail injury while the other extracts had no effect. However, by studying tissue regeneration in this tail injury model, we showed that only *A. triplinervis* and *H. lanceolatum* aqueous extracts improved this process, whereas it was either reduced or unchanged after the treatment with the remaining plant extracts. Interestingly, all the aqueous extracts of the study displayed potent effects in limiting lipid accumulation in an overfeeding model of larvae.

### *4.1. Polyphenol content/composition and acute toxicity of the aqueous extracts*

It is well-known that medicinal plants are rich and natural sources of bioactive phytochemicals including polyphenols which are largely responsible for their beneficial capacities [52,61]. In this work, we first characterized the total polyphenolic contents of 11 plant aqueous extracts. We found a total polyphenol content ranging between 14.2 and 103.2 mg GAE/g of dried plants. Using the same extraction method (infusion), the amounts found in our extracts were closed to those reported in previous studies for *A. borbonica*, *A. triplinervis*, *H. lanceolatum* and *S. cumini* extracts [7,14,20,50,59]. The differences observed, for example in the case of *A. theiformis* extract (22 mg GAE/g in our study *vs* 72 mg GAE/g in Checkouri *et al.* study) can be explained by the use of a different raw material as well as a different grinding method leading to a different particle size and then to a different extraction yield [26]. The most common classification divides phenolics into two main categories: flavonoids (such as anthocyanins, flavanols, flavanones, flavonols,

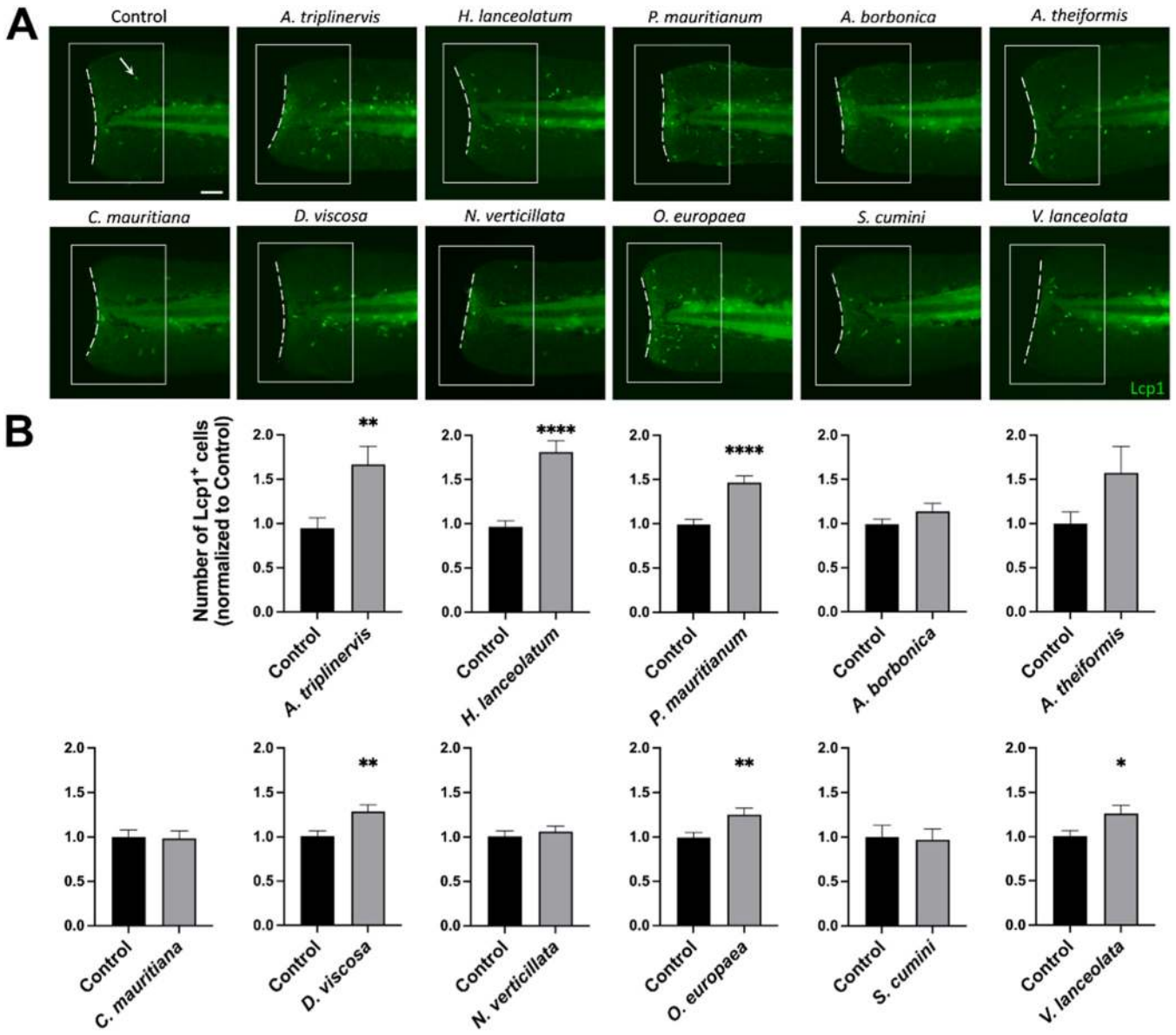



**Fig. 4. Effects of 11 medicinal plant extracts on recruitment of macrophages after tail injury. (A)** Representative pictures of tail amputations of 3-dpf eleutheroembryos, incubated with E3 medium (Control) and the plant aqueous extract at the respective MNTCs for 6 h followed by Lcp1 immunostaining. **(B)** Normalized number of Lcp1-positive (macrophages) cells (arrows) at the injury site (white box). Dotted lines represent the tail amputation site. Results are expressed as means ± SD (n = 30/group from 3 independent experiments). *p < 0.05, **p < 0.01, ****p < 0.0001 (Student *t*-test). Bar = 60 μm.

flavones, and isoflavones) and non-flavonoids (including phenolic acids, xanthones, stilbenes, lignans, and tannins) [47]. The biological properties of these molecules are closely linked to their bioavailability (absorption, distribution, metabolism, and excretion within the body) as well as their chemical structure (structure-activity relationship), which is influenced by various factors, such as the number of hydroxyl groups, the presence and position of additional functional groups, and the degree of polymerization [17,24]. By high-resolution mass spectrometry (LC-MS/MS), we found that the aqueous extracts were abundant in phenolic acids and flavonoids including chlorogenic acid (*H. lanceolatum* extract), gallic acid (*P. mauritianum* and *S. cumini* extracts), ferulic acid (*A. triplinervis* extract), quercetin (*O. europaea* extracts), epicatechin and procyanidin (*D. viscosa* extract) and mangiferin (*A. theiformis* extract). Previous phytochemical analyses also reported these major polyphenols in reunionese traditional plants [7,14,20,59]. Specific coumarins in *A. triplinervis* (ayapanin and ayapin) as well as tannins (mostly ellagitannins) in *S. cumini* were also identified [14,51].

The consumption of medicinal plants registered at the French Pharmacopoea is generally considered to be safe. However, a chronic consumption of polyphenol-rich products can lead to adverse effects including hepatotoxicity and nephrotoxicity [31]. In this context, we investigated for the first time the toxicity of the plant aqueous extracts in zebrafish eleutheroembryos according to the OECD guidelines (0–96 hpf) and using another toxicity test (3–5dpf) in which detoxification organs (liver and kidney) are functional and early embryonic toxicity is avoided. Interestingly, for some aqueous extracts (*A. borbonica*, *C. mauritiana*, *O. europaea* and *V. lanceolata*), 5-dpf eleutheroembryos were less vulnerable to toxic compounds than 0-96-hpf eleutheroembryos. This is probably due to the ability of eleutheroembryos from 3-dpf to metabolize, detoxify or excrete some toxins thanks to functional detoxification organs (liver and kidneys) at this developmental stage [12,15]. As well, we can speculate that early embryonic toxicity is limited in this test, as at 3dpf morphogenesis is already well-established. In contrast, for *A. theiformis* and *S. cumini*, the aqueous extracts appeared

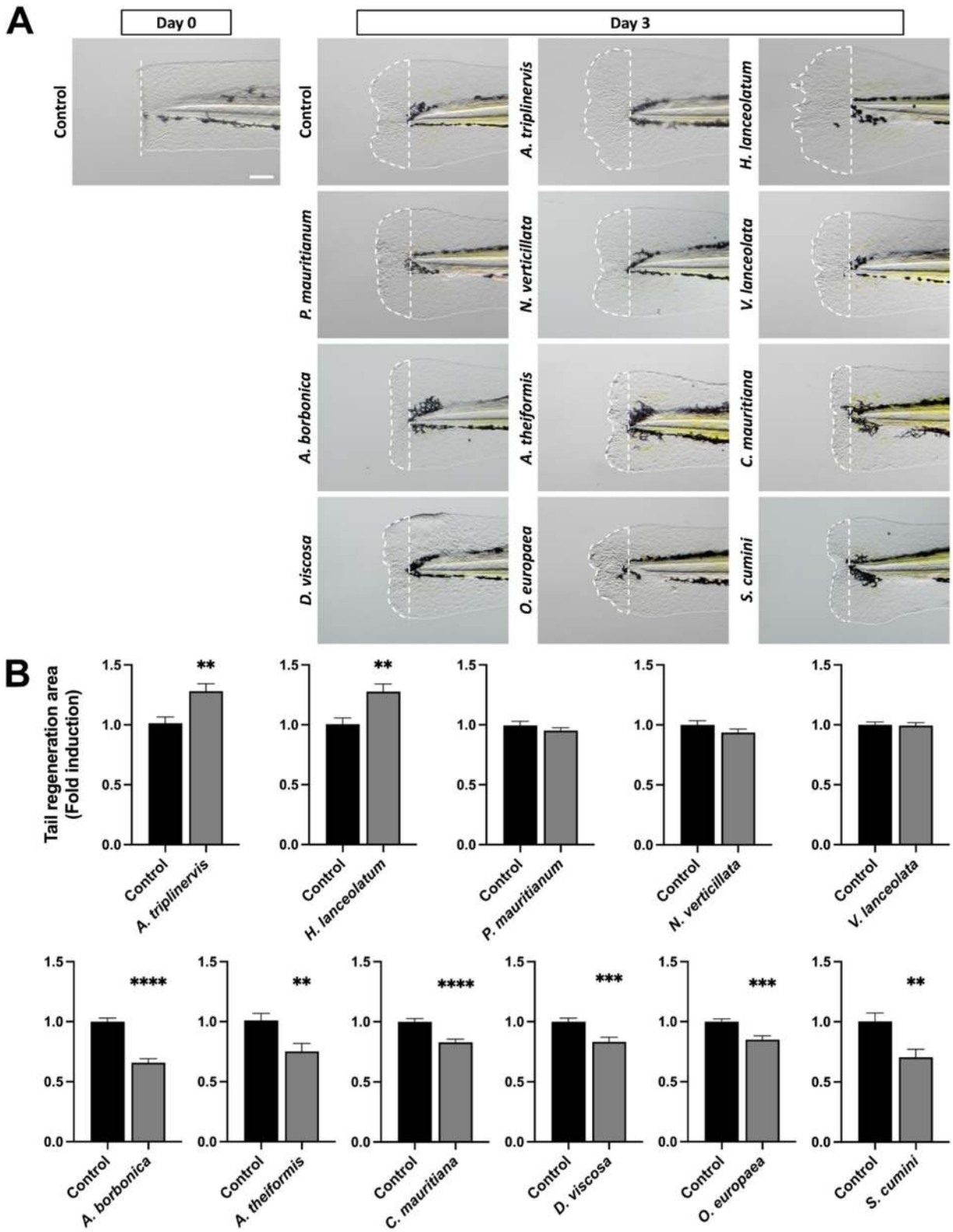



**Fig. 5. Effects of 11 medicinal plant extracts on tissue regeneration after tail amputation in zebrafish eleutheroembryos. (A)** Representative pictures of eleutheroembryos incubated in E3 medium (Control) and the plant aqueous extract at the respective MNTCs, at 0- and 3-days post-amputation (dotted) and **(B)** measurement of tail regeneration area 3-days post-amputation. Results are expressed as means ± SD (n = 22–49/group from 3 independent experiments). $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ (Student *t*-test). Bar = 38 μm.

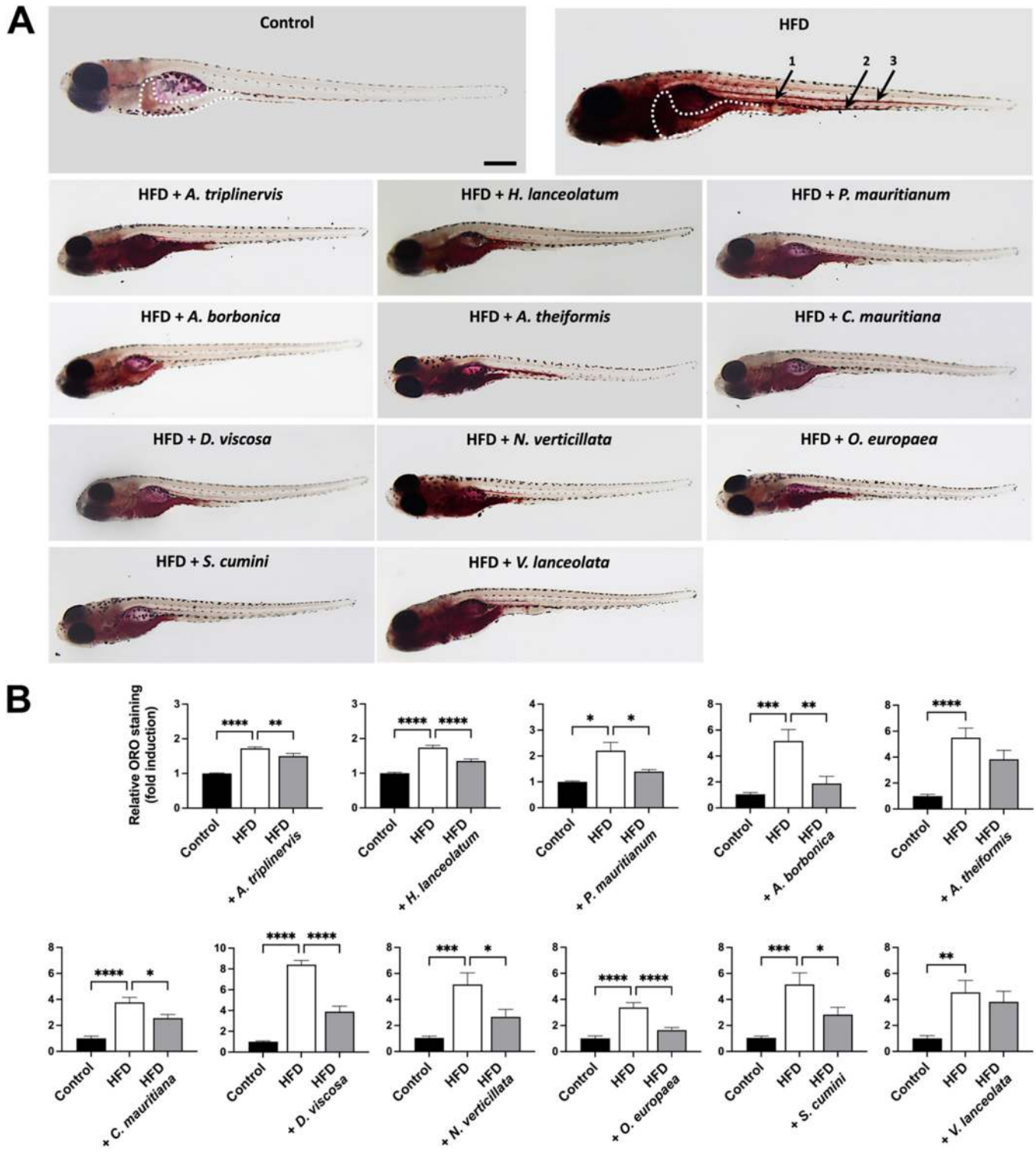



**Fig. 6. Effects of 11 medicinal plant extracts on lipid accumulation in HFD larvae. (A)** Representative pictures and **(B)** quantification of ORO staining of normally fed larvae (Control), overfed (HFD) and overfed larvae treated with the plant aqueous extract at their respective MNTCs (HFD + "plant aqueous extract"). Dotted lines indicate the ORO staining in the digestive tract. Arrows show the staining in vessels including the posterior cardinal vein (1), caudal vein (2) and dorsal aorta (3). Results are expressed as means ± SD (n = 25–48 larvae/group from 3 independent experiments). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ (One-way ANOVA). Bar = 300 µm.

to be more toxic on 5-dpf eleutheroembryos than 96-hpf eleutheroembryos which can be explained by the fact that from 0 to 72-hpf, the chorion can be considered as an extra physiological barrier protecting zebrafish embryo until hatching [44]. According to these tests, the most toxic aqueous extracts (with lower $LC_{50}$) were not necessarily the richest in polyphenols and the toxic effects could be caused more by the combination of polyphenols or by other minor molecules present in the different extracts. In many studies, the supraphysiological administration of some polyphenols (e.g., chlorogenic acid, ferulic acid, quercetin and mangiferin) can result in altered hepatic and renal functions as well

as carcinogenic effects [10,13,23,35]. However, toxicity assessment of medicinal plants is important for the evaluation of their safety and help for investigating new medical alternative approaches. Finally, based on the OECD toxicity tests, we determined the respective MNTCs for each plant extract that did not cause mortality or exhibit toxic effects on hepatic, cardiac, renal and nervous systems.

### 4.2. Effects of the aqueous extracts on immune cell recruitment and tail regeneration

Based on traditional knowledge, the selected plants are recommended for their anti-inflammatory activities [32]. In this context, we tested the inflammatory response and regeneration in zebrafish following tail amputation [36]. Our results showed that *A. borbonica*, *A. triplinervis*, *D. viscosa*, *H. lanceolatum*, *O. europaea*, *P. mauritianum* and *V. lanceolata* aqueous extracts increased the number of macrophages and neutrophils at the injury site 6 h after the amputation whereas *A. theiformis*, *C. mauritiana*, *N. verticillata* and *S. cumini* aqueous extracts had no impact on the recruitment of immune cells. Inflammation is important in regenerative processes that can occur over several days [37]. Therefore, we also evaluated the effects of the plant aqueous extracts on tail regeneration after 3 days of treatment. Unexpectedly, in our experimental conditions, most of our plant extracts impaired or had no effect on the tail regeneration, although they improved immune cell recruitment. Based on the same model, *A. triplinervis* and *H. lanceolatum* aqueous extracts have been reported to increase immune cell recruitment as well as tissue regeneration [14,20]. Considering the estimated polyphenol content at the respective MNTCs, it is important to notice that some aqueous extracts contained almost 4–6 times more or 3 times less polyphenols (GAE) than *A. triplinervis*, *H. lanceolatum* and *P. mauritianum* extracts (around 32 mg) which can probably have an impact on tail regeneration. Such difference can also be related to distinct polyphenolic compositions between all the plant aqueous extracts. Indeed, phytochemical analyses by high resolution-mass spectrometry showed the presence of chlorogenic acid, ferulic acid, quercetin/kaempferol and derivatives which were more concentrated in the pro-regenerative aqueous extracts than the others. Many studies reported their pro-repair properties acting among others, on macrophage polarization (switching from a pro- "M1" to an anti-inflammatory "M2" phenotype), redox status recovery, cell proliferation (angiogenesis, epithelialization), collagen synthesis and matrix remodeling [5,8,28,41,63]. In this context, although immune cells were efficiently recruited at the injury site, it can also be assumed that the other aqueous extracts might have negative effects on these other processes involved in tissue repair. However, it is important to note that in our experimental conditions, tail regeneration was not complete after 3 days of treatment. Studying the entire regeneration process (up to 1 week) could therefore provide additional data on the plant effects. As well, it would be interesting to study the impact of these extracts on angiogenesis occurring during tail repair, that could affect the regenerative processes.

### 4.3. Effects of the aqueous extracts on metabolic disorders

Herbal medicine and in particular, polyphenol consumption has been associated with the prevention of chronic diseases [6,54]. Traditional medicinal plants from Réunion Island are also supposed to limit obesity and diabetes [32,53]. Besides their immunomodulatory and regenerative properties, we investigated the potential effect of the plant aqueous extracts in a zebrafish overfeeding model, as previously described [20]. The overfeeding resulted in a lipid accumulation in the digestive tract and blood vessels of larvae which was consistent with previous studies [20,65]. Surprisingly, the treatment of overfed larvae with the aqueous extracts from almost all the selected plants (except for *A. theiformis* and *V. lanceolata* aqueous extracts) induced a significant decrease in ORO staining. The mechanisms sustaining these lipid-lowering effects could be related to a lower food intake or improved lipid metabolism and excretion. It has been suggested that polyphenols were able to modulate appetite-related factors and therefore mediate the food intake and satiety [43]. Recently, performing a DIO model in adult zebrafish, the treatment with *A. borbonica* or *P. mauritianum* aqueous extracts resulted in a marked reduction in weight gain without affecting feeding behavior of overfed fish [21,22]. These effects were associated with a decrease in liver steatosis and an increased production of feces by the DIO fish treated with *P. mauritianum* compared to untreated and control groups [21]. In obese mice, *A. borbonica* limited the metabolic disorders related to obesity including insulin resistance, hyperglycemia, hyperinsulinemia and dyslipidemia [57]. Administration of other plant extracts such as *O. europaea* leaf extract and *S. cumini* seed powder also lead to anti-obesity properties in rodents by reducing body weight gain, improving the inflammatory status in liver and adipose tissue, preventing microbiota dysbiosis and vascular dysfunction associated with obesity [58,60]. In addition, many studies have focused on the specific role of individual polyphenolic compounds and have shown their efficiency to limit detrimental effects associated with obesity (i.e. adipose tissue expansion, inflammation, oxidative stress and insulin-sensitivity) [11,16,39]. Chlorogenic acid has been reported to improve body fat distribution and lower fatty acid and cholesterol biosynthesis in obese mice by suppressing the activities of fatty acid synthase, HMG-CoA reductase and ACAT in the liver whereas up-regulating fatty acid oxidation and PPARα expression [9]. In general, flavonoids also modulate factors involved in lipogenesis, lipolysis, expenditure energy, fatty acids β-oxidation, inflammatory responses and oxidative stress [29]. *In vitro*, exposure of preadipocytes to quercetin resulted in attenuated adipogenesis with the diminution of adipogenesis-related factors and enzymes (SREBP-1, C/EBPα, PPARγ and FAS) [2]. Other studies have suggested that ellagitannins (abundant in *S. cumini*) attenuated dyslipidemia and insulin resistance [27]. Finally, knowing the impact of our aqueous extracts on such mechanisms could be interesting to better characterize the anti-obesogenic (i.e. adipose tissue expansion, lipid accumulation, inflammation, oxidative stress and insulin-sensitivity) activities of these traditional plants. In this line, Mangiferin that is abundant in *A. theiformis* was already demonstrated to induce brown adipogenesis and promote thermogenesis in brown adipocytes suggesting that this compound may ameliorate obesity via activation of brown adipose tissue [48]. Its administration to obese mice had a positive effect on body weight, insulin-sensitivity, glucose and lipid metabolism, as well as on liver steatosis, and adipocyte hypertrophy [39]

### 4.4. Limitations of the zebrafish model and study findings

The development of disease models is important for understanding the physiopathology of many disorders and establishing therapeutic strategies. In this context, zebrafish has rapidly emerged as a robust model for translational biomedical research. High homology to human genome and physiological processes supports the use of zebrafish as a reliable non-mammalian model [25,38]. Indeed, more than 70 % of human genes have a zebrafish orthologue. However, many zebrafish genes are duplicated, raising the question of their functions and how they may affect physiology [46]. In this work, we used zebrafish for studying the toxicity and the therapeutic potential of different plant extracts. However, like any experimental models, zebrafish has several limitations which require further consideration. For example, fish are ectothermic organisms which can probably act on their behavioral, metabolic and neuroendocrine regulation. Furthermore, the method of administering plant extracts by immersion in zebrafish may present certain limitations. Unlike traditional human use, where herbal teas are taken in the diet, absorption through the skin in zebrafish leads to a different metabolism of the active molecules. In addition, a small proportion may be ingested with a different composition of the intestinal microbiota [34], leading to a different metabolism of polyphenols in particular.

Limitations also arise from the nature of the material being tested.

The aqueous extracts used in this study are complex mixtures. Although we identified several polyphenolic compounds that are likely to be involved in the observed effects, we cannot rule out the contribution of other bioactive or synergistic molecules. The precise mechanisms of action remain to be fully elucidated, particularly with regard to the signaling pathways involved in the beneficial effects. Additionally, all experiments were performed over short durations (a few days), which does not reflect the chronic exposure that would occur during human use.

While zebrafish do not replace preclinical rodent models, they provide a valuable *in vivo* system with good predictive value. It would be interesting to corroborate such effects in preclinical mouse models regarding oral or intravenous administration. Further investigations are needed to isolate active constituents, assess long-term effects and confirm the therapeutic relevance of these findings in mammalian models.

## 5. Conclusion

In this work, we reported the toxic thresholds of *A. borbonica*, *A. theiformis*, *A. triplinervis*, *C. mauritiana*, *D. viscosa*, *H. lanceolatum*, *N. verticillata*, *O. europaea*, *P. mauritianum*, *S. cumini* and *V. lanceolata* aqueous extracts in zebrafish eleutheroembryos.

This study, assessing acute toxicity, enabled us to define the MNTCs for each plant extract that did not alter the expression of biomarkers involved in hepatic, cardiac, renal and cerebral functions, as well as endocrine signalling during developmental stages. We showed the beneficial potential of some plant extracts on the recruitment of immune cells and tissue regeneration. Importantly, most of the aqueous extracts (except *A. theiformis* and *V. lanceolata* aqueous extracts) displayed potent lipid-lowering properties in an overfeeding zebrafish model. The use of zebrafish represents an important step in the study of the health benefits of medicinal plants. Further investigations using preclinical rodent models as well as the use of individual molecules could be of interest in order to validate the properties of the plant extracts studied in mammals. The choice of studying preparations already used in humans also makes it possible to envisage nutritional studies to validate the beneficial properties of infusions in clinically-monitored trials. Overall, this study has highlighted the use of medicinal plants from Réunion Island in the prevention and management of metabolic disorders such as obesity. The scientific approach developed in this research as well as the zebrafish models could become a go-no-go study towards preclinical testing in pathological mouse models.

## Author contributions

LG, OM, BV, J-LB and ND designed the experiments. LG, DF, TAA, MLA, AH, BG and ND performed the experiments. MB and BV have been involved in toxicity assay. BV also participated in the analysis and control of LC-MS/MS data. All the authors participated in the analysis of the experiments and/or in the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

## Funding

This work was supported by Grants from the University of La Réunion, European Union (EU) Région Réunion-French State national counterpart: FEDER RE0022527 (ZEBRATOX).

## CRediT authorship contribution statement

**Olivier Meilhac:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Investigation, Conceptualization. **Bryan Veeren:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Matthieu Bringart:** Writing – review & editing, Resources, Investigation. **Batoul Ghaddar:** Writing – review & editing, Investigation. **Anaïs Hoarau:** Writing – review & editing, Investigation. **Apalama Marie-Laurine:** Writing – review & editing, Investigation. **Tatiana Al Anaissy:** Writing – review & editing, Investigation. **Danielle Fernezelian:** Writing – review & editing, Writing – original draft, Validation, Investigation, Conceptualization. **Laura Gence:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Nicolas Diotel:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Jean-Loup Bascands:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of Competing Interest

In the name of all my coauthors, I declare that all of us declare no conflict of interests.

## Acknowledgements

We thank Dr Sepand Rastegar for providing the transgenic *Tg(GFAP::GFP)* zebrafish line. We also thank our collaborators for proofreading the manuscript. We thank Akinao-lab for their services in flavonoid quantification and mass spectrometry.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2025.118305.

## Data availability

Data will be made available on request.

## References

[1] Agence nationale de sécurité du médicament et des produits de santé (2021) La Pharmacopée française.
[2] J. Ahn, H. Lee, S. Kim, J. Park, T. Ha, The anti-obesity effect of quercetin is mediated by the AMPK and MAPK signaling pathways, Biochem. Biophys. Res. Commun. 373 (2008) 545–549.
[3] A.N.M. Alamgir, Herbal Drugs: Their Collection, Preservation, and Preparation; Evaluation, Quality Control, and Standardization of Herbal Drugs, in: A.N. M. Alamgir (Ed.), Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1: Pharmacognosy, Springer International Publishing, Cham, 2017, pp. 453–495.
[4] N. Atale, C.B. Mishra, S. Kohli, R.K. Mongre, A. Prakash, S. Kumari, U.C.S. Yadav, R. Jeon, V. Rani, Anti-inflammatory effects of S. cumini seed extract on gelatinase-B (MMP-9) regulation against hyperglycemic cardiomyocyte stress, Oxid. Med. Cell Longev. 2021 (2021) 8839479.
[5] D. Bagdas, B.C. Etoz, Z. Gul, S. Ziyanok, S. Inan, O. Turacozen, N.Y. Gul, A. Topal, N. Cinkilic, S. Tas, M.O. Ozyigit, M.S. Gurun, In vivo systemic chlorogenic acid therapy under diabetic conditions: wound healing effects and cytotoxicity/genotoxicity profile, Food Chem. Toxicol. Intern. J. Published British Industrial Biol. Res. Assoc. 81 (2015) 54–61.
[6] M. Boccellino, S. D'Angelo, Anti-obesity effects of polyphenol intake: current status and future possibilities, Int. J. Mol. Sci. 21 (2020).
[7] E. Checkouri, F. Reignier, C. Robert-Da Silva, O. Meilhac, Evaluation of polyphenol content and antioxidant capacity of aqueous extracts from eight medicinal plants from reunion island: protection against oxidative stress in red blood cells and preadipocytes, Antioxidants (2020) 9.
[8] W.C. Chen, S.S. Liou, T.F. Tzeng, S.L. Lee, I.M. Liu, Effect of topical application of chlorogenic acid on excision wound healing in rats, Planta Med. 79 (2013) 616–621.
[9] A.S. Cho, S.M. Jeon, M.J. Kim, J. Yeo, K.I. Seo, M.S. Choi, M.K. Lee, Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice, Food Chem. Toxicol. Intern. J. Published British Indus. Biol. Res. Assoc. 48 (2010) 937–943.
[10] M. Dimitrov, I. Nikolova, N. Benbasat, G. Kitanov, N. Danchev, Acute toxicity, antidepressive and mao inhibitory activity of mangiferin isolated from hypericum aucheri, Biotechnol. Biotechnol. Equip. 25 (2011) 2668–2671.

[11] P.V. Dludla, B.B. Nkambule, B. Jack, Z. Mkandla, T. Mutize, S. Silvestri, P. Orlando, L. Tiano, J. Louw, S.E. Mazibuko-Mbeje, Inflammation and oxidative stress in an obese state and the protective effects of gallic acid, Nutrients 11 (2018).

[12] I.A. Drummond, A.J. Davidson, Zebrafish kidney development, Methods Cell Biol. 134 (2016) 391–429.

[13] W.Y. Du, C. Chang, Y. Zhang, Y.Y. Liu, K. Sun, C.S. Wang, M.X. Wang, Y. Liu, F. Wang, J.Y. Fan, P.T. Li, J.Y. Han, High-dose chlorogenic acid induces inflammation reactions and oxidative stress injury in rats without implication of mast cell degranulation, J. Ethnopharmacol. 147 (2013) 74–83.

[14] D. Fernezelian, L. Gence, M. Bringart, B. Veeren, Y. Bedoui, O. Meilhac, J.-L. Bascands, N. Diotel, Ayapana triplinervis Vahl: potential toxicity and therapeutic effects assessed in a zebrafish model, Phytomedicine 3 (2023) 100384.

[15] H.A. Field, E.A. Ober, T. Roeser, D.Y. Stainier, Formation of the digestive system in zebrafish. I. Liver morphogenesis, Dev. Biol. 253 (2003) 279–290.

[16] L.A. Forney, N.R. Lenard, L.K. Stewart, T.M. Henagan, Dietary quercetin attenuates adipose tissue expansion and inflammation and alters adipocyte morphology in a tissue-specific manner, Int. J. Mol. Sci. 19 (2018).

[17] C.G. Fraga, M. Galleano, S.V. Verstraeten, P.I. Oteiza, Basic biochemical mechanisms behind the health benefits of polyphenols, Mol. Asp. Med. 31 (2010) 435–445.

[18] G.R. Garcia, P.D. Noyes, R.L. Tanguay, Advancements in zebrafish applications for 21st century toxicology, Pharmacol. Ther. 161 (2016) 11–21.

[19] M. Gemberling, T.J. Bailey, D.R. Hyde, K.D. Poss, The zebrafish as a model for complex tissue regeneration, Trends Genet 29 (2013) 611–620.

[20] L. Gence, D. Fernezelian, M. Bringart, B. Veeren, A. Christophe, F. Brion, O. Meilhac, J.L. Bascands, N. Diotel, Hypericum lanceolatum lam. medicinal plant: potential toxicity and therapeutic effects based on a zebrafish model, Front. Pharm. 13 (2022) 832928.

[21] B. Ghaddar, L. Gence, B. Veeren, M. Bringart, J.L. Bascands, O. Meilhac, N. Diotel, Aqueous extract of psiloxylon mauritianum, rich in gallic acid, prevents obesity and associated deleterious effects in zebrafish, Antioxidants 11 (2022).

[22] B. Ghaddar, B. Veeren, P. Rondeau, M. Bringart, C. Lefebvre d'Hellencourt, O. Meilhac, J.L. Bascands, N. Diotel, Impaired brain homeostasis and neurogenesis in diet-induced overweight zebrafish: a preventive role from A. borbonica extract, Sci. Rep. 10 (2020) 14496.

[23] M. Harwood, B. Danielewska-Nikiel, J.F. Borzelleca, G.W. Flamm, G.M. Williams, T.C. Lines, A critical review of the data related to the safety of quercetin and lack of evidence of in vivo toxicity, including lack of genotoxic/carcinogenic properties, Food Chem. Toxicol. Intern. J. Published British Indus. Biol. Res. Assoc. 45 (2007) 2179–2205.

[24] K.E. Heim, A.R. Tagliaferro, D.J. Bobilya, Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships, J. Nutr. Biochem. 13 (2002) 572–584.

[25] K. Howe, M.D. Clark, C.F. Torroja, J. Torrance, C. Berthelot, M. Muffato, J. E. Collins, S. Humphray, K. McLaren, L. Matthews, S. McLaren, I. Sealy, M. Caccamo, C. Churcher, C. Scott, J.C. Barrett, R. Koch, G.J. Rauch, S. White, W. Chow, B. Kilian, L.T. Quintais, J.A. Guerra-Assunção, Y. Zhou, Y. Gu, J. Yen, J. H. Vogel, T. Eyre, S. Redmond, R. Banerjee, J. Chi, B. Fu, E. Langley, S.F. Maguire, G.K. Laird, D. Lloyd, E. Kenyon, S. Donaldson, H. Sehra, J. Almeida-King, J. Loveland, S. Trevanion, M. Jones, M. Quail, D. Willey, A. Hunt, J. Burton, S. Sims, K. McLay, B. Plumb, J. Davis, C. Clee, K. Oliver, R. Clark, C. Riddle, D. Elliot, G. Threadgold, G. Harden, D. Ware, S. Begum, B. Mortimore, G. Kerry, P. Heath, B. Phillimore, A. Tracey, N. Corby, M. Dunn, C. Johnson, J. Wood, S. Clark, S. Pelan, G. Griffiths, M. Smith, R. Glithero, P. Howden, N. Barker, C. Lloyd, C. Stevens, J. Harley, K. Holt, G. Panagiotidis, J. Lovell, H. Beasley, C. Henderson, D. Gordon, K. Auger, D. Wright, J. Collins, C. Raisen, L. Dyer, K. Leung, L. Robertson, K. Ambridge, D. Leongamornlert, S. McGuire, R. Gilderthorp, C. Griffiths, D. Manthravadi, S. Nichol, G. Barker, S. Whitehead, M. Kay, J. Brown, C. Murnane, E. Gray, M. Humphries, N. Sycamore, D. Barker, D. Saunders, J. Wallis, A. Babbage, S. Hammond, M. Mashreghi-Mohammadi, L. Barr, S. Martin, P. Wray, A. Ellington, N. Matthews, M. Ellwood, R. Woodmansey, G. Clark, J. Cooper, A. Tromans, D. Grafham, C. Skuce, R. Pandian, R. Andrews, E. Harrison, A. Kimberley, J. Garnett, N. Fosker, R. Hall, P. Garner, D. Kelly, C. Bird, S. Palmer, I. Gehring, A. Berger, C.M. Dooley, Z. Ersan-Ürün, C. Eser, H. Geiger, M. Geisler, L. Karotki, A. Kirn, J. Konantz, M. Konantz, M. Oberländer, S. Rudolph-Geiger, M. Teucke, C. Lanz, G. Raddatz, K. Osoegawa, B. Zhu, A. Rapp, S. Widaa, C. Langford, F. Yang, S.C. Schuster, N.P. Carter, J. Harrow, Z. Ning, J. Herrero, S.M. Searle, A. Enright, R. Geisler, R.H. Plasterk, C. Lee, M. Westerfield, P.J. de Jong, L.I. Zon, J.H. Postlethwait, C. Nüsslein-Volhard, T.J. Hubbard, H. Roest Crollius, J. Rogers, D.L. Stemple, The zebrafish reference genome sequence and its relationship to the human genome, Nature 496 (2013) 498–503.

[26] A.A. Jovanović, V.B. Đorđević, G.M. Zdunić, D.S. Pljevljakušić, K.P. Šavikin, D. M. Gođevac, B.M. Bugarski, Optimization of the extraction process of polyphenols from Thymus serpyllum L. herb using maceration, heat- and ultrasound-assisted techniques, Sep. Purif. Technol. 179 (2017) 369–380.

[27] I. Kang, T. Buckner, N.F. Shay, L. Gu, S. Chung, Improvements in metabolic health with consumption of ellagic acid and subsequent conversion into urolithins: evidence and mechanisms, Adv. Nutr. 7 (2016) 961–972.

[28] V. Kant, B.L. Jangir, V. Kumar, A. Nigam, V. Sharma, Quercetin accelerated cutaneous wound healing in rats by modulation of different cytokines and growth factors, Growth Factors 38 (2020) 105–119.

[29] M. Kawser Hossain, A. Abdal Dayem, J. Han, Y. Yin, K. Kim, S. Kumar Saha, G. M. Yang, H.Y. Choi, S.G. Cho, Molecular mechanisms of the anti-obesity and anti-diabetic properties of flavonoids, Int. J. Mol. Sci. 17 (2016) 569.

[30] B. Knap, D. Nieoczym, U. Kundap, K. Kusio-Targonska, W. Kukula-Koch, W. A. Turski, K. Gawel, Zebrafish as a robust preclinical platform for screening plant-derived drugs with anticonvulsant properties—a review, Front. Mol. Neurosci. 16 - 2023 (2023).

[31] J.D. Lambert, S. Sang, C.S. Yang, Possible controversy over dietary polyphenols: benefits vs risks, Chem. Res. Toxicol. 20 (2007) 583–585.

[32] R. Lavergne, Tisaneurs et plantes médicinales indigènes: l'île de La Réunion, Orphie, 2001.

[33] G. Loupit, J.V. Fonayet, S. Prigent, D. Prodhomme, A.S. Spilmont, G. Hilbert, C. Franc, G. De Revel, T. Richard, N. Ollat, S.J. Cookson, Identifying early metabolite markers of successful graft union formation in grapevine, Hortic. Res 9 (2022).

[34] H. Lu, P. Li, X. Huang, C.H. Wang, M. Li, Z.Z. Xu, Zebrafish model for human gut microbiome-related studies: advantages and limitations, Med. Microecol. 8 (2021) 100042.

[35] C. Mancuso, R. Santangelo, Ferulic acid: pharmacological and toxicological aspects, Food Chem. Toxicol. Intern. J. Published British Indus. Biol. Res. Assoc. 65 (2014) 185–195.

[36] V. Miskolci, J. Squirrell, Distinct inflammatory and wound healing responses to complex caudal fin injuries of larval zebrafish, Elife 8 (2019).

[37] R.A. Morales, M.L. Allende, Peripheral macrophages promote tissue regeneration in zebrafish by fine-tuning the inflammatory response, Front. Immunol. 10 (2019) 253.

[38] M. Nguyen, E. Yang, N. Neelkantan, A. Mikhaylova, R. Arnold, M.K. Poudel, A. M. Stewart, A.V. Kalueff, Developing 'integrative' zebrafish models of behavioral and metabolic disorders, Behav. Brain Res. 256 (2013) 172–187.

[39] J.W. Noh, H.Y. Lee, B.C. Lee, Mangiferin ameliorates obesity-associated inflammation and autophagy in high-fat-diet-fed mice: in silico and in vivo approaches, Int. J. Mol. Sci. 23 (2022).

[40] OCDE (2013) *Test No. 236: Fish Embryo Acute Toxicity (FET) Test.*

[41] Y. Özay, S. Güzel, Ö. Yumrutaş, B. Pehlivanoğlu, İ. Erdoğdu, H, Z. Yildirim, B. A. Türk, S. Darcan, Wound healing effect of kaempferol in diabetic and nondiabetic rats, J. Surg. Res. 233 (2019) 284–296.

[42] S.K. Pal, Y. Shukla, Herbal medicine: current status and the future, Asian Pac. J. Cancer Prev. 4 (2003) 281–288.

[43] K.S. Panickar, Effects of dietary polyphenols on neuroregulatory factors and pathways that mediate food intake and energy regulation in obesity, Mol. Nutr. Food Res. 57 (2013) 34–47.

[44] S.P.P. Pereira, D. Boyle, A. Nogueira, R.D. Handy, Differences in toxicity and accumulation of metal from copper oxide nanomaterials compared to copper sulphate in zebrafish embryos: delayed hatching, the chorion barrier and physiological effects, Ecotoxicol. Environ. Saf. 253 (2023) 114613.

[45] M.C. Picot, M.F. Mahomoodally, Effects of Aphloia theiformis on key enzymes related to diabetes mellitus, Pharm. Biol. 55 (2017) 864–872.

[46] J. Postlethwait, A. Amores, A. Force, Y.-L. Yan, Chapter 8 The Zebrafish Genome, in: H.W. Detrich, M. Westerfield, L.I. Zon (Eds.), Methods in Cell Biology, Academic Press, 1998, pp. 149–163.

[47] S. Prabhu, A. Molath, H. Choksi, S. Kumar, R. Mehra, Classifications of polyphenols and their potential application in human health and diseases, Int. J. Physiol. Nutr. Phys. Educ. 6 (2021) 293–301.

[48] M.S. Rahman, Y.S. Kim, Mangiferin induces the expression of a thermogenic signature via AMPK signaling during brown-adipocyte differentiation, Food Chem. Toxicol. 141 (2020) 111415.

[49] M. Rajasekar, S. Mohan, Advancements in zebrafish model for assessing the toxicological effects of polyphenolic (flavonoid) compounds, Results Chem. 16 (2025) 102371.

[50] S. Silva, L. Gomes, F. Leitão, A.V. Coelho, L.V. Boas, Phenolic compounds and antioxidant activity of Olea europaea L. fruits and leaves, Food Sci. Technol. Int. 12 (2006) 385–395.

[51] B. Singh, J.P. Singh, A. Kaur, N. Singh, Insights into the phenolic compounds present in jambolan (Syzygium cumini) along with their health-promoting effects, Int. J. Food Sci. Technol. 53 (2018) 2431–2447.

[52] S. Škrovánková, L. Mišurcová, L. Machů, Antioxidant activity and protecting health effects of common medicinal plants, Adv. Food Nutr. Res. 67 (2012) 75–139.

[53] J. Smadja, C. Marodon, Le grand livre des plantes médicinales de l'île de La Réunion: inscrites à la pharmacopée française, Éditions Orphie, 2016.

[54] A. Sofowora, E. Ogunbodede, A. Onayade, The role and place of medicinal plants in the strategies for disease prevention, Afr. J. Tradit. Complement Alter. Med 10 (2013) 210–229.

[55] G. Sudhakaran, S. Gopi, S. Priya, R. Pachaiappan, I. Soundharrajan, B.O. Almutairi, J. Arockiaraj, Apigenin regulates bisphenol-a induced polycystic ovarian syndrome (PCOS) symptoms in-vivo zebrafish, Reprod. Sci. (2025).

[56] J. Taïlé, A. Arcambal, P. Clerc, A. Gauvin-Bialecki, M.P. Gonthier, Medicinal plant polyphenols attenuate oxidative stress and improve inflammatory and vasoactive markers in cerebral endothelial cells during hyperglycemic condition, Antioxidants 9 (2020).

[57] J. Taïlé, M. Bringart, C. Planesse, J. Patché, P. Rondeau, B. Veeren, P. Clerc, A. Gauvin-Bialecki, S. Bourane, O. Meilhac, D. Couret, M.P. Gonthier, Antioxidant polyphenols of antirhea borbonica medicinal plant and caffeic acid reduce cerebrovascular, inflammatory and metabolic disorders aggravated by high-fat diet-induced obesity in a mouse model of stroke, Antioxidants (2022) **11**.

[58] A. Ulla, M.A. Alam, B. Sikder, F.A. Sumi, M.M. Rahman, Z.F. Habib, M. K. Mohammed, N. Subhan, H. Hossain, H.M. Reza, Supplementation of Syzygium cumini seed powder prevented obesity, glucose intolerance, hyperlipidemia and oxidative stress in high carbohydrate high fat diet induced obese rats, BMC Complement. Altern. Med. 17 (2017) 289.

[59] B. Veeren, B. Ghaddar, M. Bringart, S. Khazaal, M.P. Gonthier, O. Meilhac, N. Diotel, J.L. Bascands, Phenolic profile of herbal infusion and polyphenol-rich extract from leaves of the medicinal plant antirhea borbonica: toxicity assay determination in zebrafish embryos and larvae, Molecules 25 (2020).
[60] T. Vezza, A. Rodríguez-Nogales, F. Algieri, J. Garrido-Mesa, M. Romero, M. Sánchez, M. Toral, B. Martín-García, A.M. Gómez-Caravaca, D. Arráez-Román, A. Segura-Carretero, V. Micol, F. García, M.P. Utrilla, J. Duarte, M.E. Rodríguez-Cabezas, J. Gálvez, The metabolic and vascular protective effects of olive (Olea europaea L.) leaf extract in diet-induced obesity in mice are related to the amelioration of gut microbiota dysbiosis and to its immunomodulatory properties, Pharmacol. Res. 150 (2019) 104487.
[61] X. Wang, Y. Qi, H. Zheng, Dietary polyphenol, gut microbiota, and health benefits, Antioxidants 11 (2022).
[62] World Health Organization, WHO global report on traditional and complementary medicine 2019, World Health Organization, 2019.
[63] D.J. Yang, S.H. Moh, D.H. Son, S. You, A.W. Kinyua, C.M. Ko, M. Song, J. Yeo, Y. H. Choi, K.W. Kim, Gallic acid promotes wound healing in normal and hyperglucidic conditions, Molecules 21 (2016).
[64] L. Zang, L.A. Maddison, W. Chen, Zebrafish as a model for obesity and diabetes, Front. Cell Dev. Biol. 6 (2018) 91.
[65] J. Zhou, Y.Q. Xu, S.Y. Guo, C.Q. Li, Rapid analysis of hypolipidemic drugs in a live zebrafish assay, J. Pharmacol. Toxicol. Methods 72 (2015) 47–52.