Evaluation of *Myrcia bella* in murine osteosarcoma cells: Effect of the extract and enriched fractions of tannins and flavonoids

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Abstract

Myrcia bella Cambess (Myrtaceae) is an important and common plant, native to the Brazilian Cerrado, with cytotoxicity, antimicrobial, and antidiabetic properties. Therefore, the effects of crude hydroalcoholic extract (CE) and fractions of ellagitannins (ELT) and flavonoids (FV) from *Myrcia bella* leaves were evaluated in a UMR-106 murine osteosarcoma cells and MC3T3 (normal cell). Cell viability and migration, production of reactive oxygen species (ROS) and matrix metalloproteinase (MMP) -2 and -9 activities were evaluated. In general, CE (80 µg/mL), ELT (160 µg/mL) and FV (64 µg/mL) reduced cell viability ($p<0.05$). FV (64 µg/mL) was more effective in inhibition of cell migration, ROS production, and MMP-2 activity when compared to CE and ELT. *Myrcia bella* a rich source of phenolic compounds and its fraction of flavonoids have cytotoxic effects on osteosarcoma cells, preserving the viability of normal osteoblasts. Due to its antioxidant capacity, flavonoid may be a new therapeutic strategy for cancer.

Keywords: *Myrcia bella*; Medicinal plants; Cell viability; Osteosarcoma; Flavonoids.

1. Introduction

Osteosarcoma is the most frequent primitive malignant bone tumor, especially in adolescents and young adults worldwide, with an aggressive growth in the primary sites and metastasizes to other organs. The significant side effects of chemopreventative drugs and the development of resistance to chemotherapy seriously compromises treatment efforts. Thus, is essential the development of new drugs for the treatment of osteosarcoma (Liu et al. 2017).

In recent years, medicinal plants have been extensively used due to their anticarcinogenic and chemoprotective potential. The advantage in using these plants is due their less toxic antineoplastic, antitumor and antiproliferative properties when compared with allopathic therapies (Gezici and Sekeroglu 2019). Promising results of plant crude extracts has led to the identification of its active ingredients responsible for their biological and pharmacological activities (Machado et al. 2016).

Myrcia bella Cambess (Myrtaceae) is an important and common plant, native to the Brazilian Cerrado. Pharmacological studies have demonstrated the cytotoxicity, antimicrobial (Santos et al. 2018), and antidiabetic (Vareda et al. 2014) properties of the hydroalcoholic leaves extracts. And phenolic compounds were related to bioactivity in all these studies.

Phytochemical studies of the hydroalcoholic leaves extract of *Myrcia bella* revealed several acetylated flavonoid derivatives of quercetin and myricetin (Saldanha et al. 2013), in addition to tannins, derived from ellagic acid and gallic acid (Saldanha et al. 2020).

Despite of the phytochemical and biological investigations of *M. bella*, there is still a lack of more comprehensible studies that identify the active class of compounds against tumor cells. Do these compounds have an effect in tumor cells? Thus, in the present study, the effects of crude hydroalcoholic extract (CE) and fractions of ellagitannins and flavonoids from *M. bella* leaves were evaluated in a UMR-106 murine osteosarcoma cells.

2. Results and Discussion

2.1. *Myrcia bella* extract and fractions decrease cellular viability of UMR-106

In general, the compounds promoted a reduction in cell viability, especially of tumor cells (UMR-106), with increased concentration, with emphasis on the crude extract (CE) and the flavonoid (FV) fraction. This cellular viability reduction was more specific (about 2-fold) to UMR-106 when to compare with MC3T3-E1 cells. UMR-106 cells, when in contact with the 64 µg/mL FV fraction in the 48h period, are already compromised, probably activating the cell death mechanism.

The analysis of MTT reduction demonstrated that the UMR-106 strain showed decreased viability in the 24h and 48h periods for the highest concentrations of extract and fractions when compared to the control group (no treatment), and in the 72h period, there was an even more significant decrease in cell viability. In addition, when compared to the MC3T3-E1 control line, the decrease in viability is greater for the tumor line, showing statistical differences ($p<0.05$).

In relation to crystal violet both strains suffer decreased of cellular viability in the highest concentrations of extract and fractions in the period of 48 hours, as well as in the reduction of MTT. The results presented here demonstrated that the crude extract and the flavonoid fraction induced cytotoxicity of the UMR-106 lineage.

2.2. Hematoxylin-eosin staining

Regarding the cellular morphology of the UMR-106, compared to the control group, the cells treated with CE have a decrease in the nuclei and the shape has irregularities. With the ELT there was an increase in size and the cells are more widely spaced than in the control; with FV the cells showed a slight decrease in nucleus, apparently presenting more cytoplasm of cells in the image than an entire cell, no longer presenting a standard morphology.

Regarding the cell morphology of MC3T3-E1, compared to the control group, it appears that the CE did not change the morphology. In the ELT treated group, the cells appear to be more stretched, but this is also a small difference compared to the control. The group of cells treated with FV showed a morphological alteration and apparently cell degradation occurred.

The FV fraction apparently promoted a reduction in the number of cells in both cell lines. However, this reduction and morphological alterations, were more characteristic in UMR-106 than in MC3T3-E1.

2.3. Efficacy of the flavonoid fraction in inhibiting cell migration

In the evaluation of the inhibition of the migratory activity of osteosarcoma cells, the CE and FV were more effective. The results obtained allow us to observe that after the incubation time of 24 hours in the concentrations of CE 80µg/mL, ELT 160µg/mL and FV 64µg/mL, the cells closed the wound by 10.61%, 13.25% and 13.15% respectively, compared to the zero hour. In 48 hours, in the same concentrations of CE, ELT and FV described, there was closure in 18.70%, 25.33% and 15.15% of the wound, respectively. Within 72 hours, the concentrations of CE, ELT and FV caused closure in 25.46%, 39.44% and 18.95% of the wound, respectively. For the control, in the periods of 24h, 48h and 72h, the wound closure was 30.19%, 37.01% and 53.31%, respectively compared to the zero hour.

Flavonoids were effective in inhibiting cell migration. The result of CE was also high, probably because it is related to the amount of FV present in its portion. Both results our, cytotoxicity and migration, were in the same direction of other paper, that showed treatment with myricetin promoting inhibition of cellular proliferation and apoptosis in canine osteosarcoma (Park et al. 2018). Another point, better detailed to next topic, are MMPs activities and them relation with cellular migration. MMPs are involved with tumoral cell migration (Tokuhara et al. 2019) and some plants extracts could reduce these activities (Matos et al. 2019), and, maybe, decrease a cell migration.

2.4. Gelatin Zymography assay

The fraction FV 64 µg/mL had an effect a reduction in MMP-2 and MMP-9 activities, thus, showing no significant differences in relation to the control (no treatment) ($p>0.05$).

The degradation of the extracellular matrix (MEC) is very crucial during metastasis (Do Thi and Hwang 2014). Other reports (Berndt et al. 2004; Li et al. 2019) show that the inhibitory effect of quercetin on cell migration and invasion in human osteosarcoma cells has therapeutic effects against osteosarcoma. Lan et al. (2017) noted that treatment with higher concentrations of quercetin resulted in minor increases in tumor growth when HOS and MG63 cells were treated with 100 µM quercetin, and a definite decrease in tumor growth was observed. Taken together, these findings suggest that quercetin plays a crucial role in mitigating osteosarcoma metastases. Our findings seem to converge with these authors, since quercetin is a component of the MB extract, and we detected a tendency to reduce in the activity of MMP-9 in the cells

treated with MB. Additionally, tendency to inhibit activity of MMP-2 and 9 resulting from our tests, possibly may have been caused by the action of the flavonoids quercetin and myricetin present in MB, due to their antioxidant properties (Serpeloni et al. 2015).

Therefore, due to the chemical composition of *M.bella* and taking into account the diverse potentials that heterosides flavonoids have in relation to a wide range of activities in a murine osteosarcoma strain, such as reduced cell viability, inhibition of cell migration, decreased production of ROS and showed a reduced activity of MMP-2 and tending to the reduction of MMP-9 activity, *M. bella* becomes a promising source of studies in the face of a wide range of biological activities.

3. Conclusions

This is the first study to evaluate the effects of *Myrcia bella* on murine osteosarcoma cells (in vitro), and it has shown that the flavonoid fraction effectively showed an antioxidant activity, inhibition of migration and cytotoxic effect on the UMR-106 cell line (osteosarcoma), preserving the viability of normal osteoblasts. In short, due to its high phenolic content and antioxidant activities, *Myrcia bella* can be seen as a potential source of new natural antioxidants.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgment

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Supplementary Materials

Evaluation of *Myrcia bella* in murine osteosarcoma cells: Effect of the extract and enriched fractions of tannins and flavonoids

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Abstract

Myrcia bella Cambess (Myrtaceae) is an important and common plant, native to the Brazilian Cerrado, with cytotoxicity, antimicrobial, and antidiabetic properties. Therefore, the effects of crude hydroalcoholic extract (CE) and fractions of ellagitannins (ELT) and flavonoids (FV) from *Myrcia bella* leaves were evaluated in a UMR-106 murine osteosarcoma cells and MC3T3 (normal cell). Cell viability and migration, production of reactive oxygen species (ROS) and matrix metalloproteinase (MMP) -2 and -9 activities were evaluated. In general, CE (80 µg/mL), ELT (160 µg/mL) and FV (64 µg/mL) reduced cell viability ($p<0.05$). FV (64 µg/mL) was more effective in inhibition of cell migration, ROS production, and MMP-2 activity when compared to CE and ELT. *Myrcia bella* a rich source of phenolic compounds and its fraction of flavonoids have cytotoxic effects on osteosarcoma cells, preserving the viability of normal osteoblasts. Due to its antioxidant capacity, flavonoid may be a new therapeutic strategy for cancer.

Keywords: *Myrcia bella*; Medicinal plants; Cell viability; Osteosarcoma; Flavonoids.

1. Experimental

1.1. Plant Material

Samples of *Myrcia bella* Cambess. leaves were collected in the Municipal Botanical Garden of Bauru (22°20’30" S 49°00'30.4" W) in February 2018. Fertile branches were used for identification and deposited at the Herbarium of UNESP in Bauru (UNBA) under code number 5508. Fresh leaves were hot air dried at 40°C for 48 h. The access and shipment of component of genetic heritage, as issued by the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq), was performed under authorization No. 010468/2014-51 of Genetic Heritage Management Council (Conselho de Gestão do Patrimônio Genético – CGEN).

1.1.2. Extraction

Powdered leaves (1.3 kg) were submitted to percolation using EtOH:H_2O 7:3% v/v at room temperature. The obtained solution was concentrated to dryness under reduced pressure at 40 °C and lyophilized yielding 364 g of the hydroalcoholic leaves extract.

1.1.3. HPLC-PAD analysis

HPLC-PAD analysis was performed using a HPLC system PU-2089S Plus (Jasco®, Tokyo, Japan) coupled to a photodiode array-detector MD 2015 Plus (Jasco®, Tokyo, Japan). The chromatographic separations were conducted using a Phenomenex® Luna C18 column (250 x 4.6 mm id, 5µm). The eluent was monitored by means of UV absorbance measured at 254 and 366 nm and the UV spectra were recorded between 190 and 600 nm. This method was used for the profiling of the main components present in the fractions.

1.1.4. Fractionation of the hydroalcoholic leaves extract using medium pressure liquid chromatography (MPLC)

The hydroalcoholic leaves extract (3 g) was fractionated by MPLC (Buchi, Flawil, Switzerland) equipped with two pumps model C-601. Chromatographic separations were performed using a dry packed column with 90 g of Discovery® DSC-C_{18} as the stationary phase (40−63 µm, Sigma-Aldrich, St. Louis, USA). The gradient of elution using a solvent system with MeOH (B) and H_2O (A) (+0.1% formic acid). The elution was performed using a gradient as follows: 5-15% of B in 300 min; 15-100% of B in 120 min. The flow rate was 7.5 mL/min. These conditions were first optimized on an analytical HPLC column (250 × 4.6 mm i.d., 5 µm)

(Guillarme et al. 2007; Guillarme et al. 2008). Two fractions were obtained, tannin fraction (g) (1.1 g) and flavonoid fraction (0.9 g) (FV).

1.2. Cell Culture

For the cell expansion, mouse pre-osteoblast MC3T3-E1, acquired commercially by the *American Type Culture Collection (*ATCC® - CRL-2593) and rat osteoblast-like osteosarcoma UMR-106 (ATCC® - CRL-1661) were grown, respectively, in Minimum Essential Medium Eagle - Alpha Modification (α-MEM) (Gibco, Thermo-Scientific) and Dulbecco's Modified Eagle Medium – high glucose (DMEM) (Sigma-Aldrich), supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin 10.000 UI/streptomycin 0.060 g/L (Gibco) (Cavagis et al. 2014; Oliveira et al. 2017). For subculture, the cells were incubated with trypsin (0.25%) for 5 minutes at 37°C, followed by trypsin inactivation with medium containing 10% FBS. After centrifugation at 1,200 rpm for 5 minutes, the pellet was resuspended in the respective media and the cells were cultured in bottles for further experiments. Cells were incubated at 37ºC in a humid atmosphere containing 5% CO_2 (Oliveira et al. 2016). All cell culture plastic ware was obtained from Greiner Bio-One (Frickenhausen Germany).

1.2.1. MC3T3-E1 Differentiation

The MC3T3-E1 cell control is a murine pre-osteoblast strain and a tumor strain (UMR-106) is osteoblast. In order to compare both cell line, MC3T3-E1 differentiation with osteogenic medium (α-MEM + 10% SBF culture medium plus 50 μg/mL of ascorbic acid and 10mM β-glycerophosphate) were induced for 4 days (Quarles et al. 1992).

1.3. Cell viability assays

1.3.1. MTT reduction

Cell viability data was obtained through the analysis of mitochondrial activity, carried out with the MTT reduction method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma-Aldrich).

UMR-106 were seeded at a density of $5x10^3$ cells/well in 96-well plate (TPP®) and incubated for 48h for adhesion. For MC3T3-E1 experiments, the cells were seeded at a density of $5x10^2$ cells/well in 96-well plate, kept for 24h at 37 °C and them differentiated with osteogenic medium for 4 days. After the adhesion and differentiation period, the culture medium was replaced by the medium containing different concentration of *M. bella*, established by previous experiments and by the solubility of each one, Crude Extract (CE) (10, 20, 40 and 80 μg/mL); Ellagitannins (ELT) (20, 40, 80 and 160 μg/mL) and Flavonoids (FV) (8, 16, 32

and 64 µg/mL) in addition to the Control group (medium + NaOH 0.5 mol/L). These NaOH concentration (0.5 mol/L) was established to allow the extracts solubilization and was used to all experiment. The cells were washed with phosphate-buffered saline (PBS) and incubated with MTT solution (0.5 mg/mL) for 4 hours at 37 °C. The MTT solution was then replaced with dimethyl sulfoxide (DMSO); Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) for 30 min. The absorbance was determined at 550 nm using a microplate reader (Matos et al. 2019; Cavagis et al. 2014) (Synergy MXBased Monochromator, Bio-Tek, Winooski, VT, USA).

1.3.2. Crystal Violet

The crystal violet assay is responsible for staining the nucleic acids of cells (Kueng et al. 1989). UMR-106 were seeded at a density of $5x10^3$ cells/well in 96-well plate (TPP®) and incubated for 48h for adhesion. For MC3T3-E1, the cells were seeded at a density of $5x10^2$ cells/well in 96-well plate, keept for 24h at 37 °C and them differentiated with osteogenic medium for 4 days. After the adhesion and differentiation period, the culture medium was replaced by the medium containing different concentration of *M. bella* Crude Extract (CE) (10, 20, 40 and 80 µg/mL); Ellagitannins (ELT) (20, 40, 80 and 160 µg/mL) and Flavonoids (FV) (8, 16, 32 and 64 µg/mL) in addition to the Control group (medium + NaOH 0.5 mol/L) for 48h. The culture medium of all the tested groups were removed and the cells were washed twice with the PBS 1X solution. The crystal violet staining solution (0.5%) was then added and incubated for 20 minutes at room temperature. After this period, a plate was washed under a tap until the crystal was completely removed. The plate was left open overnight for complete drying, methanol was added for the solubilization of crystals, and then the plate was left stirring at room temperature for 20 minutes. The absorbance was determined at 570 nm (Feoktistova et al. 2016) (Synergy™ Mx monochromator multimode microplate reader, Biotek Instruments Inc, Winooski, Vermont, USA).

1.4. Hematoxylin-eosin staining

The cells were plated at a density of $3x10^4$ cells/well for UMR-106 for 48h and for MC3T3-E1 at a density of $5x10^2$ cells/well maintained for 24 hours at 37 °C and differentiated with osteogenic medium for 4 days, both on coverslips (13 mm in diameter) in a 24-well plate. The culture medium was removed and replaced with the medium with treatments (CE 80 µg/mL, ELT 160 µg/mL, FV 64 µg/mL), in addition to the control groups with culture medium with 0.5 mol/L NaOH kept for 48h. The cells were washed with PBS 1X and fixed with 10% formalin for 30 minutes. After fixation, the wells were washed for three times with PBS 1X. Then, the cells were stained with Hematoxylin for 3 minutes and subjected to color separation

with 0.5% alcohol-acid. Eosin 1% was then added for 2 minutes, and the cells were dehydrated with an ethanol gradient, soaked with xylene and assembled with Entellan®. After staining, the cells were carefully observed using an inverted optical microscope. Representative images were captured using the digital camera *Olympus* U-TV0.5XC-3 (*Olympus*, Tokyo, Japan) microscope at 20X magnification and classified according to (Salomão et al. 2017).

1.5. Cell Migration

Cell migration is an essential phenomenon for invasion and metastasis processes. When a wound is made in the cell monolayer in the plate, cells with a high migratory capacity cross this line, making it possible to study drugs that may interfere in this mechanism (Brito et al. 2005).

The cell migration assay was based on the model described by (Andrade Carvalho et al. 2013) with adaptations. UMR-106 cells were plated at a density of $1.5x10^5$ cells/well in 12-well plates in concentrations. The cells were kept in an oven for 72 hours to acquire full confluence. Then the wells were treated with 5 µg/mL of mitomycin C (Sigma-Aldrich) for 2 hours Pretreatment with mitomycin C ensured that cells were migrating and not proliferating. The wells were washed with PBS 1X, and a vertical slit was made in the monolayer with a 1000µL tip. The wells were washed 3 times with PBS 1X, culture medium supplemented with 10% SFB, containing CE 80 µg/mL, ELT 160 µg/mL, FV 64 µg/mL and 0.5 mol/L NaOH medium without addition of compounds was used as a control. The wells were washed 3 times with PBS 1X, culture medium supplemented with 10% SFB, containing crude extract or fractions. NaOH 0.5 mol/L medium without addition of compounds was used as a control. The plates were photographed at 0 hours until 72 hours of exposure to the compounds using a phase contrast microscope coupled to the Olympus U-TV0.5XC-3 digital camera, with a 4X objective. The test was performed in biological triplicate. The percentage (%) of the slot area was calculated by the ImageJ Software and the percentage (%) of the closed slot area was calculated using the formula adapted from Rezende et al. (2015):

$$\% \text{ Slit closed area} = \frac{(\%A_0 - \%A_{72})}{\%A_0} \times 100$$

Where % A_0 is the percentage of the gap area at 0 hours and % A_{72} is the percentage of the gap area at 72 hours.

1.6. Reactive oxygen species (ROS) production analysis

UMR-106 cells were plated at a density of $3x10^4$ cells/well in 96-well microplates and incubated at 37 °C and 5% CO_2 for 24h cell adhesion. Intracellular radicals were measured by

treating them with crude extract and fractions of *M. bella* ellagitannin and flavonoid (80, 160 and 64 μg/mL respectively) or leaving them untreated (control). The cells were then loaded with 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA; Sigma-Aldrich) (Brubacher and Bols 2001). The 2′,7′-dichlorofluorescein (DCF) signal was analyzed by incubating the cells with 10 μg/mL of DCFH-DA in PBS for 30 min at 37 °C in the dark. The cells were then washed with PBS and the fluorescence intensity was measured with a spectrofluorometer at excitation at 495 nm and emission of 530 mm (monochromator based on Synergy MX) (Matos et al. 2019). The results were presented as the fluorescence intensity.

1.7. Gelatin Zymography assay

Zymography, or substrate gel electrophoresis, is a widely used non-quantitative method for assessing proteinase profiles (Leonard et al. 2018). In this study the activity of MMP-2 and MMP-9, the main group of enzymes responsible for collagen degradation were evaluated. UMR-106 cells were plated at a density of $2x10^6$ in 10 cm petri dishes and treated with CE 40, 80 μg/mL, ELT 80, 160 μg/mL, FV 32, 64 μg/mL and 0.5 mol/L NaOH medium without addition of compounds was used as a control. After the 48h period, the supernatants were collected. Protein content was quantified using the Bradford method (Bradford 1976). Protein samples (50 μg), molecular weight standards and protein metalloproteinase -2 and -9 from the human recombinant matrix (hrMMP-2 and hrMMP-9) (Calbiochem-Merck, Darmstadt, Germany) were electrophoresed in Tris chloride -10% hydrogen (HCl) polyacrylamide gels (Matsuda et al. 2014). The gels were stained with Coomassie Blue G-250 (0.1%) and digitalized using a Loccus Bio-technology DS-6000 instrument. The relative densities of gel degradation were determined by densitometry analysis using the Image J software (National Institutes of Health, NIH Image).

1.8. Statistical analysis

Data are presented as a percentage of the mean and standard deviation (SD). The parameters were analyzed by one-way ANOVA coupled with Tukey's post-hoc test; for all analyzes, values of $p<0.05$ were considered statistically significant. All statistical tests were performed using GraphPadInStat and Prism (GraphPad, San Diego, CA).

2. Results

Our previous studies with *Myrcia bella*'s leaf demonstrated the presence of flavonoid and tannin derivatives in the extract (Saldanha et al. 2020). To identify the class of compounds responsible for the antimutagenic activity, a study of the effects of the extract and fractions with

complementary composition was performed *in vitro*. To this, the crude extract and two fractions containing complementary chemical composition were prepared and evaluated using osteosarcoma cells.

2.1. Phytochemical analysis and fractionation of the active extract

In order to obtain a comprehensive view of the class of active compounds present in the 70% hydroalcoholic leaves extract, a fingerprint of the extract was obtained by HPLC-PAD and revealed the presence of flavonoids and tannins (**Fig. S1**) already described in this plant (Saldanha et al. 2013; Saldanha et al. 2020). Peaks with band at 210-270 nm (0-11 min) were attributed to the presence of tannin derivatives and bands at 240-280 and 350-380 (11-35 min) are typical UV spectra of flavonoid derivatives.

In order to identify the class of active compounds for the antimutagenic activity of *Myrcia bella*, two enriched fractions containing complementary composition of tannins and flavonoids were prepared for *in vitro* evaluation using osteosarcoma cells.

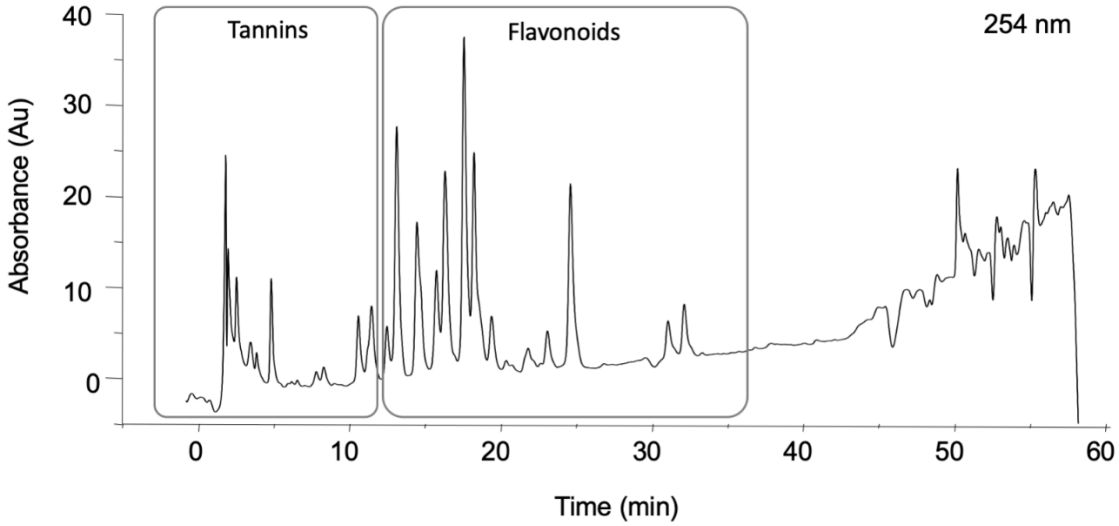

Figure S1. Chromatographic analysis of the 70% hydroalcoholic leaves extract of *Myrcia bella* obtained by HPLC-PAD. Analytical chromatogram recorded at 254 nm. For experimental condition details see MM section.

For this, a medium pressure liquid chromatography (MPLC) using silica C_{18} as stationary phase and two steps linear gradient (**Fig. S2**) was used to fractionate the 70% hydroalcoholic leaves extract and obtain two fractions, one rich in tannins and another rich in flavonoids. The HPLC-PDA analysis confirmed the presence of tannins in the polar fraction (5-15% of MeOH) (Tannin fraction) and flavonoid derivatives in the apolar fraction (15-100% of MeOH) (Flavonoid fraction).

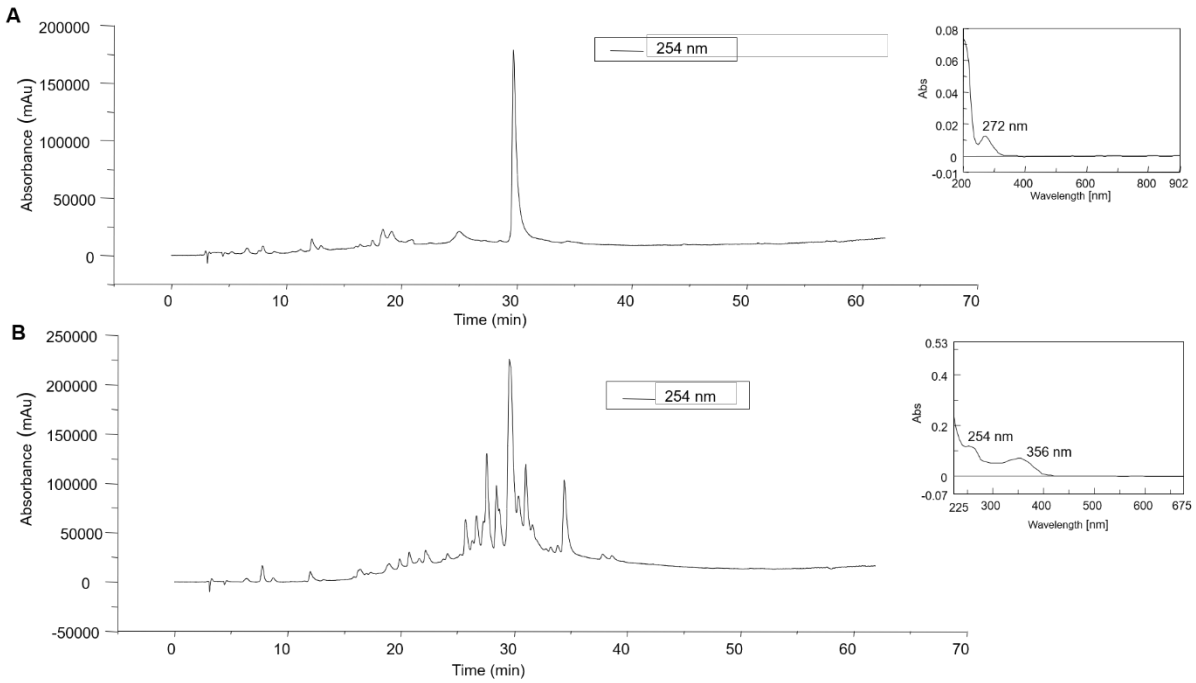

Figure S2. Supplementary material. HPLC-PAD analytical chromatogram of the fractions obtained via MPLC fractionation from the 70% hydroalcoholic leaves extract of *Myrcia bella* recorded at 254 nm. Analytical chromatograms of the tannin (**A**) and flavonoid (**B**) derivatives enriched fractions. Representative UV-vis spectra assigned to tannin and flavonoid derivatives.

2.2. *Myrcia bella* extract and fractions decrease osteosarcoma cell

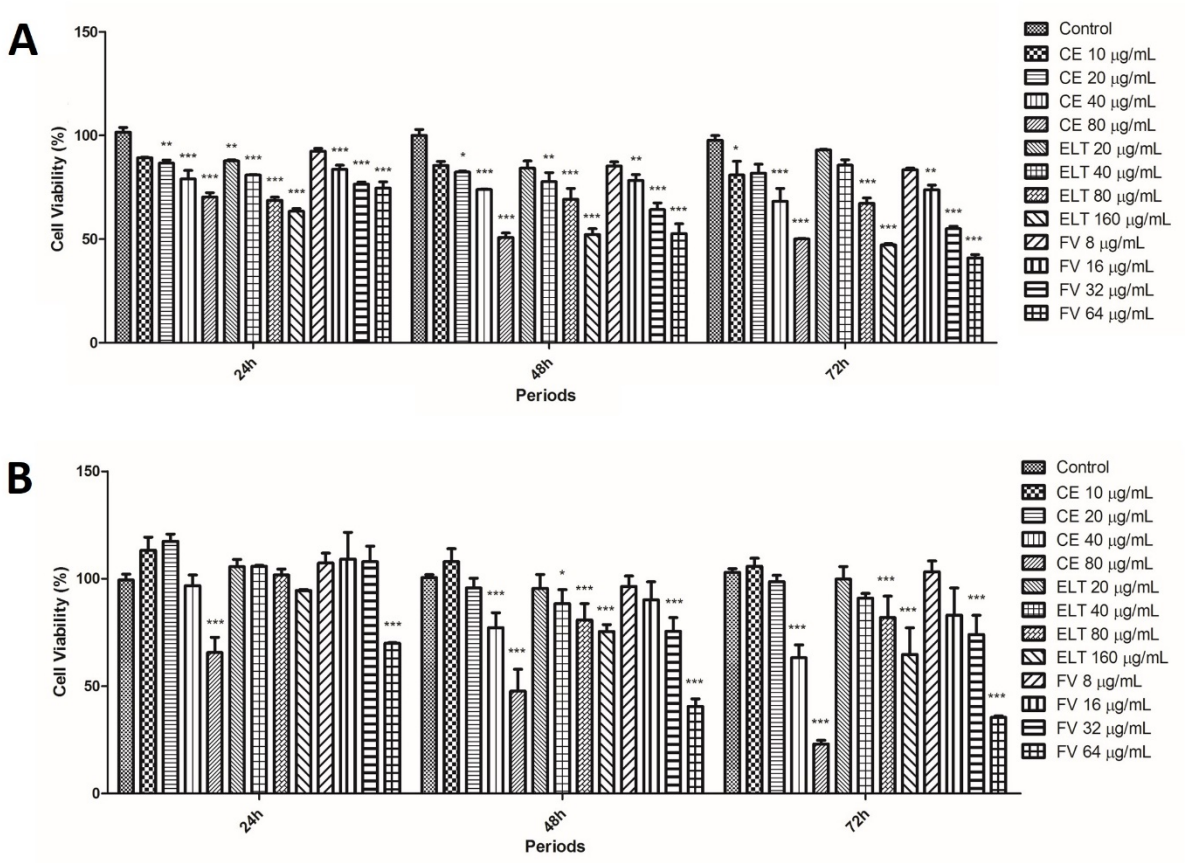

Figure S3. (**A**) The mean of the biological triplicate of the MTT reduction test. Effect of crude extract and fractions on the viability of the MC3T3-E1 strain after exposure for 24, 48 and 72h. Results express the mean ± SD. *P<0.05, ** P<0.01, *** P<0.001 represent statistical differences when compared to the control (no treatment). (CE: Crude Extract; ELT: Ellagitannin; FV: Flavonoid). (**B**) The mean of the biological triplicate of the MTT reduction test. Effect of crude extract and fractions on the viability of the UMR-106 strain after exposure for 24, 48 and 72h. Results express the mean ± SD. *P<0.05, **P<0.01, ***P<0.001 represent statistical differences when compared to the control. (CE: Crude Extract; ELT: Ellagitannin; FV: Flavonoid).

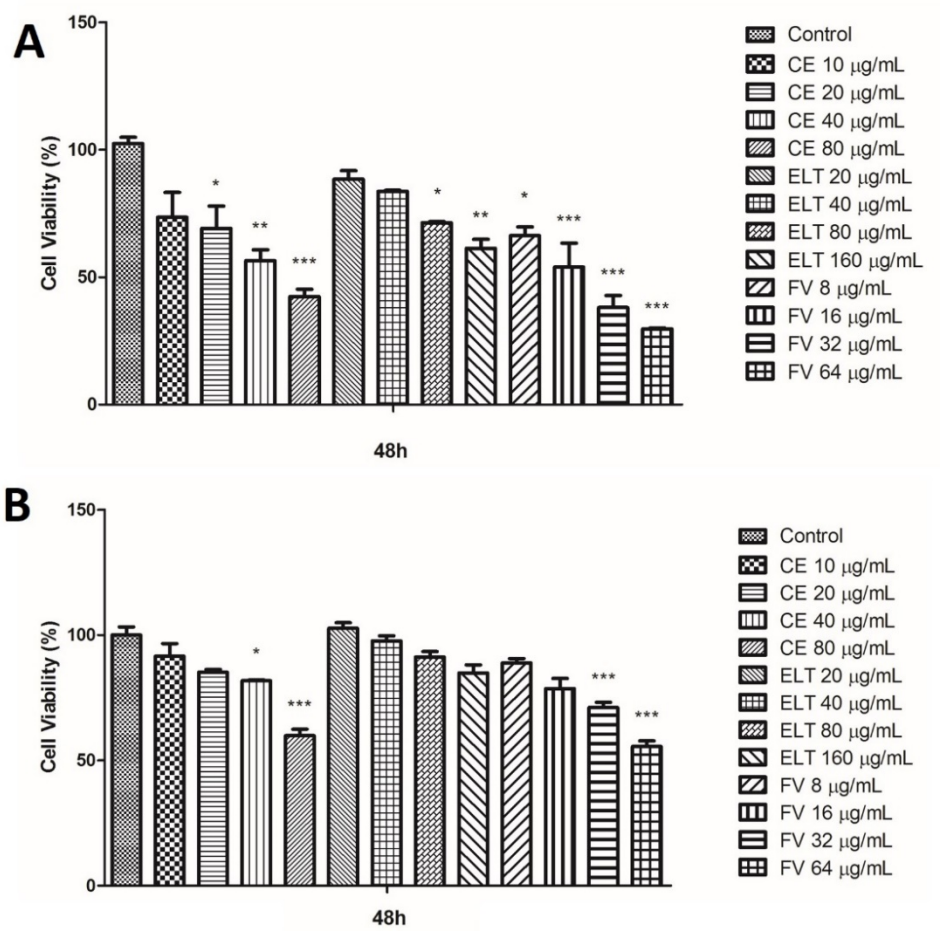

Figure S4. (**A**) The average of the biological triplicate of the Crystal Violet test. Effect of crude extract and fractions on the viability of the MC3T3-E1 strain after 48h exposure. Results express the mean ± SD. *P<0.05, **P<0.01, ***P<0.001 represent statistical differences when compared to the control (no treatment). (CE: Crude Extract; ELT: Ellagitannin; FV: Flavonoid). (**B**) The average of the biological triplicate of the Crystal Violet test. Effect of crude extract and fractions on the viability of the UMR-106 strain after 48h exposure. Results express the mean ± SD. *P<0.05, **P<0.01, ***P<0.001 represent statistical differences when compared to the control. (CE: Crude Extract; ELT: Ellagitannin; FV: Flavonoid).

2.3. Hematoxylin-eosin staining

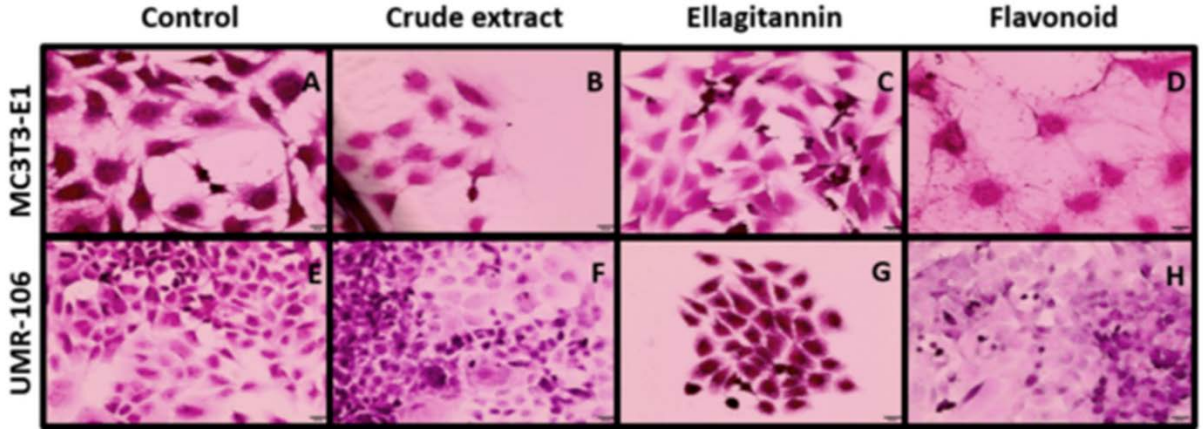

Figure S5. Microscopy of treated cell groups. Normal (differentiated MC3T3-E1) and tumor (UMR-106) osteoblasts: (**A**) MC3T3-E1 control with NaOH; (**B**) MC3T3-E1 80 µg/mL CE, (**C**) MC3T3-E1 160 µg/mL ELT, (**D**) MC3T3-E1 64 µg/mL FV, (**E**) UMR-106 control with NaOH, (**F**) UMR-106 80 µg/mL CE, (**G**) UMR-106 160 µg/mL ELT and (**H**) UMR-106 64 µg/mL FV. Hematoxylin-Eosin staining and 20X magnification.

2.4. Efficacy of the flavonoid fraction in inhibiting cell migration

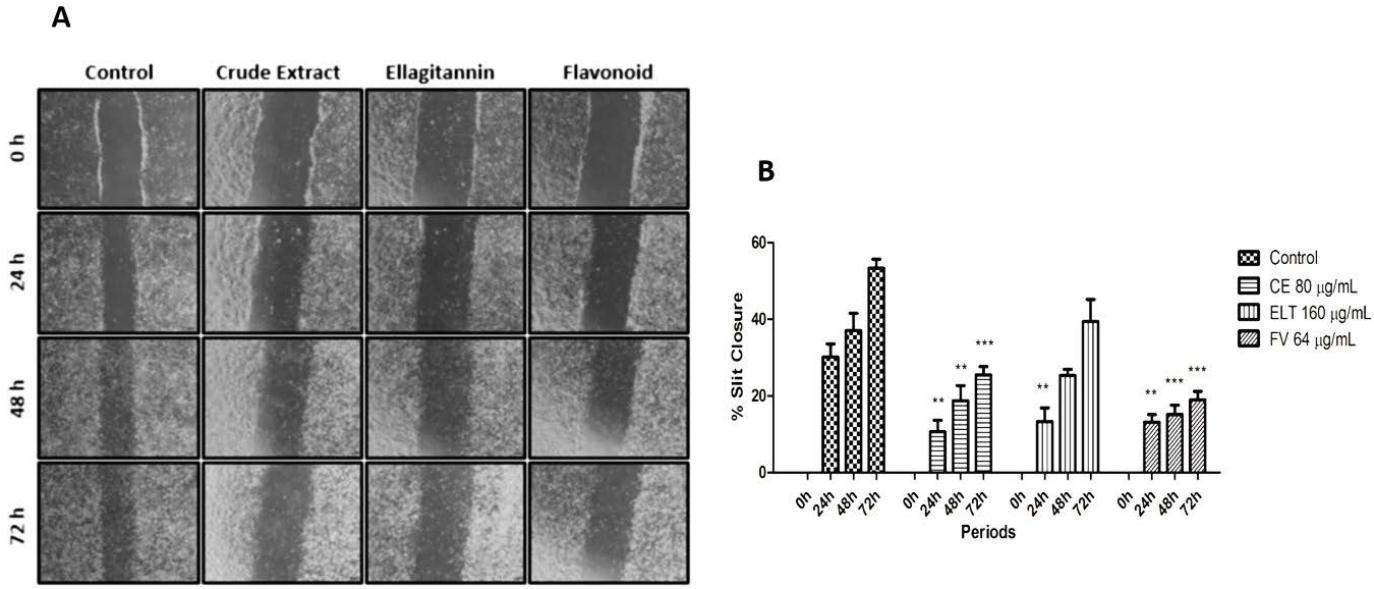

Figure S6. Representative images of the UMR-106 cell migration assay, treated or not with Crude Extract, Ellagitannin and Flavonoid fractions of Myrcia bella after the periods of 0, 24, 48 and 72 hours of treatment (**A**) compared to the Control. Graph of the percentage of wound closure (**B**). Values expressed as mean ± standard deviation. *P<0.05, **P<0.01, ***P<0.001 represent statistical differences when compared to the control (no treatment).

2.5. Flavonoids and Reactive Oxygen Species (ROS)

The concentration of FV 64 µg/mL apparently induced an antioxidant effect when compared to the control group ($p<0.05$) (**Figure S7**). There was also a reduction in fluorescence intensity in the crude extract, but without showing significant differences when compared to the control.

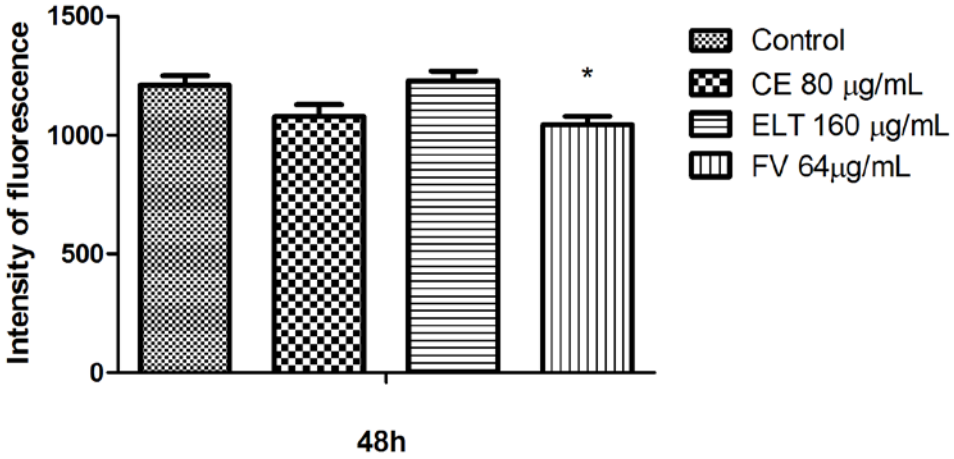

Figure S7. Production of reactive oxygen species (ROS) after exposure to *Myrcia Bella* extract. Intracellular ROS levels were measured by the DCFH-DA probe after 48 hours of treatment with crude extract and fractions. *p<0.05, compared to control (no treatment) cells.

2.6. Gelatin Zymography assay

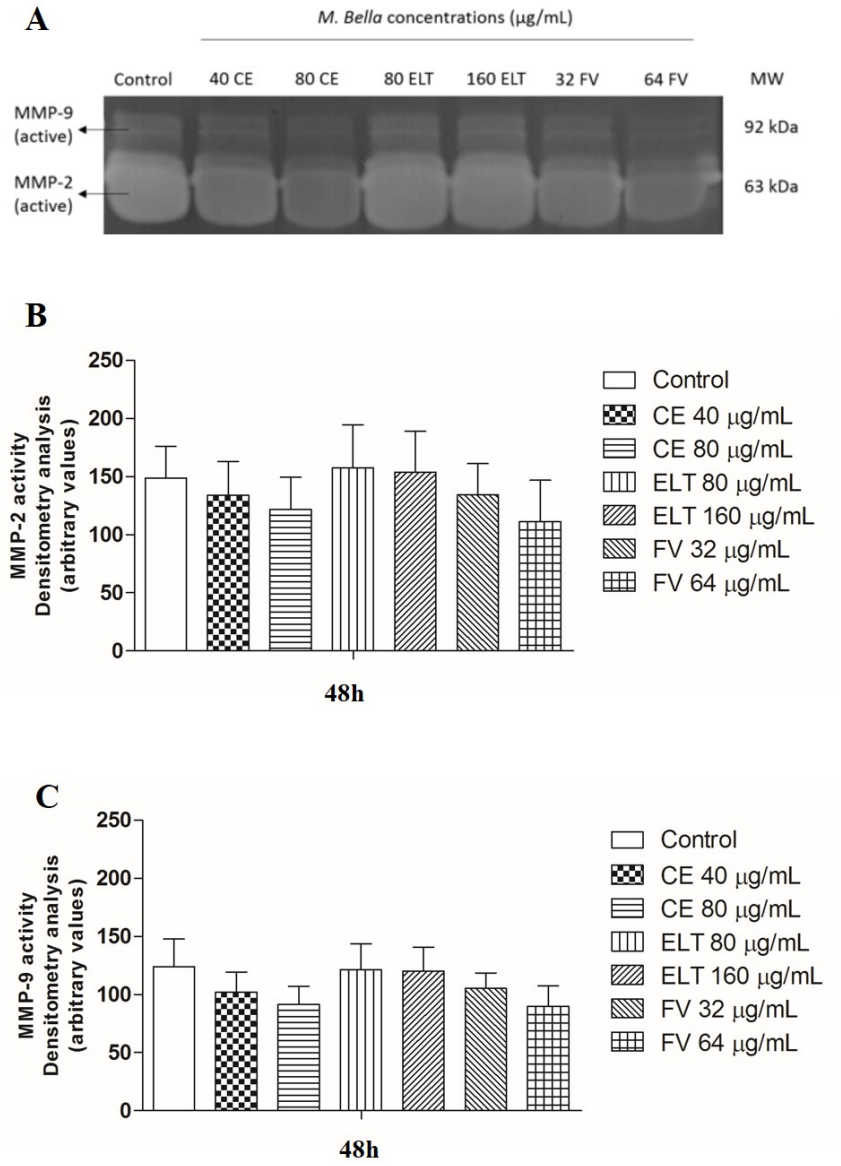

Figure S8. Effect of Myrcia bella extract and fractions on MMP-2 and MMP-9. (**A**) Gel representative of MMP-2 and 9 demonstrated by the zymography assay on 48h; (**B**) Densitometric analysis containing an arbitrary number related to active MMP-2; (**C**) Densitometric analysis containing an arbitrary number related to the active MMP-9. Data from a representative experiment conducted in triplicate are shown compared to control cells.

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