Research article

_Ficus crocata_ leaf extracts decrease the proliferation and invasiveness of breast cancer cells

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**HIGHLIGHTS**

- *F. crocata* extracts induce a cytotoxic effect on breast cancer cells.
- Ace-EFc and Dic-EFc inhibit the migration of breast cancer cells.
- Ace-EFc and Dic-EFc reduce the invasion capacity and secretion of MMP-2 and MMP-9 in MDA-MB-231 cells.
- Ace-EFc and Dic-EFc reduce actin polymerization and probably EMT in MDA-MB-231 cells.

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**ABSTRACT**

Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype due to its greater invasive capacity and non-response to hormone therapy. Several species of the _Ficus_ genus have been used as an alternative to traditional medicine against malignant diseases. Previously, leaf extracts from _Ficus crocata_ (Miq.) Mart. ex Miq. (_F. crocata_) showed antiproliferative activity in vitro against breast and cervical tumor cells without having a cytotoxic effect on non-tumor cell lines. The purpose of the study was to evaluate the effect of hexane (Hex-EFc), dichloromethane (Dic-EFc), and acetone (Ace-EFc) extracts from _F. crocata_ on the proliferative and invasive capacity of breast cancer cells MCF-7 and MDA-MB-231.

**Materials and methods:** The phytochemical profile was carried out by gas chromatography-mass spectrometry (GC-MS). Cell proliferation, migration, and invasion were determined by MTT, wound closure, and transwell assays, respectively. MMPs activity was analyzed using gelatin zymography, and fluorescence microscopy was used to visualize F-actin distribution.

**Results:** Hex-EFc, Dic-EFc, and Ace-EFc showed cytotoxic activity on MDA-MB-231 tumor cells and, to a lesser extent, on MCF-7 cells, without presenting cytotoxicity at the same concentrations in MCF-10A non-tumor cells. Dic-EFc and Ace-EFc (5–10 μg/mL) reduced the migration capacity of MCF-7 and MDA-MB-231 cells.
1. Introduction

Breast cancer is the leading cause of death from neoplasms in women worldwide (Sung et al., 2021). TNBC is characterized by a lack of expression of estrogen and progesterone receptors, as well as epidermal growth factor receptor 2 (HER2). This type of tumor does not respond to hormone therapy applied to breast cancer (Scott et al., 2019).

TNBC has a great invasive capacity because tumor cells of this subtype frequently undergo epithelial-mesenchymal transition (EMT), a significant event in regulating the motility of cancer cells (Pai et al., 2022). During EMT, high secretion of matrix metalloproteases (MMP) occurs, mainly MMP-2 and MMP-9 (Zhang et al., 2022). It has been shown that leptin, a hormone secreted by adipocytes and frequently overexpressed in overweight and obesity, induces EMT in breast cancer cells (Olea-Flores et al., 2020).

Several molecules approved as drugs are derived from natural compounds; more than 70% are used in cancer therapy, and they are considered less toxic to non-tumor cells; however, very few of these managed to reach clinical use (Seca and Pinto, 2018). Since ancient times, several Ficus species have been used in traditional medicine as a cancer preventive, cancer therapeutic, and anti-inflammatory, using their bark, roots, leaves, fruits, and latex (Lansky et al., 2008). In vitro studies have demonstrated the antitumor potential of the genus Ficus of the family Moraceae, such as Ficus altissima (Yao et al., 2021) and Ficus carica (Ou et al., 2022), among others.

Ficus crocata (Miq.) Mart. ex Miq. (F. crocata) is a species of wide distribution in southern Mexico (Ibarra-Manriquez et al., 2012). Previously, we reported that F. crocata leaf extracts decreased the number of MDA-MB-231 breast cancer cells, arresting the cell cycle and inducing apoptosis. In addition, the extract caused changes in cell morphology, mainly towards a circular phenotype, which suggested a decrease in the migration capacity of cells (Sánchez-Valdeolivier et al., 2020). In another study, Ace-EFc showed selective cytotoxic activity against the cervical cancer cell lines SiHa and HeLa without affecting non-tumor HaCaT cells; Ace-EFc even showed a cytoprotective effect against oxidative damage in non-tumor cells (De la Cruz-Concepción et al., 2021). This study aimed to compare the effect of F. crocata leaf extracts on the proliferation of breast cell lines MCF-7 (luminal A), MDA-MB-231 (TNBC), and MCF-10A (non-tumour). In addition, we assessed the effect of extracts on the migration and invasion capacity of tumor cells and the possible mechanisms by which the extracts decrease the invasive ability of cells, mainly TNBC cells.

2. Material and methods

2.1. Plant material, extraction, and phytochemical profile

Leaves of F. crocata (Miq.) Mart. ex Miq. were collected in Petaquillas, Guerrero, Mexico, and macerated sequentially with hexane, dichloromethane, and acetone as described by Sánchez-Valdeolivier et al. (2020). Subsequently, the material was filtered, and the solvents evaporated in a rotovapor at 60 °C and 80 rpm. Volatile compounds present in Hex-EFc, Dic-EFc, and Ace-EFc were identified by gas chromatography coupled with mass spectrometry (GC-MS) and analyzed as described by Sánchez-Valdeolivier et al. (2020) and De la Cruz-Concepción et al. (2021).

Interestingly, exposure to Dic-EFc and Ace-EFc (5–10 μg/mL) inhibited the invasive ability of MDA-MB-231 cells, reducing the secretion and activity of MMP-2 and MMP-9, as well as the F-actin distribution.

Conclusions: Dic-EFc and Ace-EFc at low concentrations decreased breast cancer cell proliferation and invasive-ness, mainly of MDA-MB-231 cells. The above supports the potential use of compounds from leaf extracts of F. crocata in neoadjuvant therapy to reduce the progression of breast cancer tumors, mainly triple-negative tumors.

2.2. Cell culture

MDA-MB-231 and MCF7 cells were grown in Modified Eagle Medium of Dulbecco formula 12 (DMEM/F12), supplemented with 10% fetal bovine serum (FBS) and 1% ampicillin/streptomycin. MCF10A cells were cultured in DMEM/F12 medium supplemented with 10% FBS, 10 μg/ml insulin, 0.5 μg/ml hydrocortisone, 20 ng/ml EGF and 1% antibiotics. The cells were incubated at 37 °C in an atmosphere of 5% CO₂ and 95% humidity. Cells were kept in culture up to 80% confluence.

2.3. Cell viability assay (MTT)

In a 96-well plate, 1 × 10⁴ cells per well were cultured for 24 h and posteriorly treated with 5–320 μg/mL of Hex-EFc, Dic-EFc, and Ace-EFc for 24 and 48 h. After treatment, the medium containing the extract was replaced by basal medium, and 100 μL of the MTT reagent (CT02, Millipore Corp., Bedford, MA, USA) was added for 4 h. The formazan crystals were dissolved with isopropanol, and optical density (DO) was obtained at 545/630 nm (Star Fax 2100, Awareness Technologies, Palm City, FL, USA). The percentage of viable cells was determined using the formula: Viable cells = (Ab Sample/Ab Blank) × 100. A cytotoxic effect was considered when the percentage of viable cells was below 80% concerning untreated cells. The mean inhibitory concentration (IC₅₀) was calculated using the linear equation (y = mx + b) using GraphPad Prism version 6.0 software.

2.4. Cell migration assay (scratch assay)

MDA-MB-231 and MCF7 cells were cultured in a 6-well plate to 90% confluence. Subsequently, the growth medium was replaced by a basal medium for 22 h; cytarabine-C 10 μM was added for 2 h to inhibit cell proliferation. A wound was made in the monolayer with a pipette tip and washed with PBS to eliminate the detached cells. Leptin was applied as positive control to induce cell migration, considering the conditions previously reported in the cell lines studied (50 ng/mL and 200 ng/mL for MDA-MB-231 and MCF-7 cells, respectively (Juárez-Cruz et al., 2019)). 5–20 μg/mL of Hex-EFc, Dic-EFc, and Ace-EFc extracts were applied in the presence or absence of leptin. Wound closure was monitored by taking pictures at 0, 24, and 48 h with an inverted microscope NIKON model Ts2FL. The percentage of cell migration was evaluated by measuring the wound width using the ImageJ 1.44p software.

2.5. Cell migration and invasion assays (transwell)

Inserts with a polycarbonate membrane with 8 μm pores (ECM 508) were used; 1.5 × 10⁴ MDA-MB-231 cells were seeded, and the treatment with the extracts was applied in the presence of leptin for 24 h; DMEM/F12 with 5% SFB was used as a chemoattractant. Subsequently, the insert was dried with violet crystal, and cells at the top were removed with a swab. The dye was extracted, and the DO was measured at 560 nm. The cell invasion assay was performed under the same conditions as the cell migration assay (transwell), with the difference that a protein matrix (Matrigel) was added to the top of the insert before placing the cells, and the treatments were maintained for 48 h.
2.6. Gelatin zymography

$5 \times 10^5$ cells were seeded in 60 mm plates per 24 h, synchronized for 24 h, and subsequently treated with the extracts in the presence of leptin (50 ng/mL). The supernatant was recovered and centrifugated at 3000 rpm for 40 min at 4 °C; 30 μg of protein was separated by electrophoresis in polyacrylamide gel polymerized with 1% bovine gelatin. Three washes were made with 2.5% Triton and incubated for 24 h at 37 °C with MMPs activation buffer (Tris 50 mM Tris pH 7.4, and 5 mM of CaCl$_2$). The gel was dyed with 0.25% bright Coomassie blue. Densitometric analyses of

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**Figure 1.** Phytochemical compounds identified in *F. crocata* extracts. (A) Chemical structure of the most abundant compounds. (B) NMR spectra of compounds; Hx: Hexatriacontane; Lup: Lupeol; Lac: Lup-20(29)-en-3-ol, acetate, (3β)-; Phy: Phytol; β-St: β-Sitosterol; Squ: Squalene; Vit: Vitamin-E.
white bands indicating gelatin degradation by MMPs secreted by cells were performed.

2.7. F-actin staining

5 × 10^4 cells were seeded on glass cover objects until a confluence of 70% was obtained. Dic-EFc and Ace-EFc (5–20 μg/mL) were applied in the presence of leptin for 48 h. The cells were fixed with 4% formaldehyde and permeabilized with 0.05% Triton-X100-PBS for 5 min at room temperature. Cells were incubated with rhodamine phalloidin (1:5000) for 30 min at room temperature. The images were captured on an Olympus BX43 microscope and processed with ImageJ software.

2.8. Statistical analysis

Data capture and analysis were performed using GraphPad Prism version 6.0 software. Data were expressed as mean ± standard deviation (SD). Comparisons between groups were made with a one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test. The p < 0.05 values were considered statistically significant.

3. Results

3.1. Phytochemical characterization of F. crocata leaf extracts

The most abundant compounds identified in Hex-EFc were lup-20(29)-en-3-ol acetate (3β) (28.39%), lupeol (18.61%) and hexatriacontane (9.13%). While β-sitosterol (27.86%), phytol (14.81%), and lup-20(29)-en-3-ol acetate (3β) (15.98%) were the most abundant in Dic-EFc. Finally, in Ace-EFc, the most abundant were vitamin E (42.36%), squalene (23.88%), and phytol (9.07%). Figure 1 shows the structure of the most abundant compounds (Figure 1A), and their nuclear magnetic resonance (NMR) spectra (Figure 1B). Previously, we reported the complete phytochemical profile of volatile compounds in Hex-EFc Dic-EFc (Sánchez-Valdeolivar et al., 2020) and Ace-EFc (De la Cruz-Concepción et al., 2021).

3.2. MDA-MB-231 cells are more susceptible than MCF-7 and MCF-10A cells to the cytotoxic effect induced by F. crocata extracts

High concentrations (40–320 μg/mL) of some F. crocata extracts were required to significantly decrease the number of MCF-7 and MCF-10A
viable cells (Figure 2). On the other hand, we previously reported that these *F. crocata* extracts slightly decreased the proliferation of MDA-MB-231 viable cells with low concentrations (5–20 μg/mL) and induced a cytotoxic effect with concentrations ≥80 μg/mL of extracts (Sánchez-Valdeolívar et al., 2020).

MCF-7 cells appear to be more resistant to treatment with *F. crocata* extracts, requiring concentrations around 160 μg/mL to decrease only slightly more than 20% of viable cells (Figure 2A–C), except for Ace-EFc, which showed a similar effect with ≥20 μg/mL (Figure 2C). Relevantly, Ace-EFc induced a minor cytotoxic effect in non-tumor MCF-10A cells, requiring concentrations ≥80 μg/mL to reduce the percentage of viable cells below 80% (Figure 2F). In contrast, Hex-EFc and Dic-EFc were the extracts with the most significant cytotoxic effect on non-tumor MCF10A cells because concentrations ≥80 μg/mL significantly reduced the percentage of viable cells (Figure 2D and E). The above suggests that Ace-EFc at low concentrations (5–40 μg/mL) could reduce the proliferation and viability of breast tumor cells (TNBC and Luminal A) without affecting non-tumor cells.

The mean inhibitory concentration (IC₅₀) at 48 h of each extract was calculated. In summary, MDA-MB-231 cells were more susceptible to treatment (48 h) with Dic-EFc (IC₅₀ = 40.92 ± 5.15 μg/mL) and Ace-EFc (IC₅₀ = 54.89 ± 4.99 μg/mL) followed by non-tumor MCF-10A cells (Dic-EFc IC₅₀ = 121.55 ± 5.38 μg/mL; Ace-EFc IC₅₀ = 276.84 ± 6.30 μg/mL) and MCF-7 cells (Dic-EFc IC₅₀ = 554.43 ± 3.34 μg/mL; Ace-EFc IC₅₀ = 1195.33 ± 3.66 μg/mL).

### 3.3. Ace-EFc and Dic-EFc extracts inhibit the migration capacity of MCF-7 and MDA-MB-231 cells

Exposure to Dic-EFc and Ace-EFc decreased the migration capacity of MCF-7 and MDA-MB-231 cells. Contrary to expectations, Hex-EFc increased the cell migration capacity of MDA-MB-231 cells (Figure S1); as described in the methodology, leptin was applied to induce cell migration (Figure S1). In MCF-7 cells, Hex-EFc enhanced leptin-induced cell migration (Figure 3A–C); therefore, this extract was ruled out as a possible inhibitor of migration and invasion in breast cancer cells and was excluded in the subsequent trials. In contrast, exposure to 5–10 μg/mL of Ace-EFc and Dic-EFc decreased cell migration capacity at 24 and 48 h, even in presence of leptin (Figure 3A–C). Interestingly, in MDA-MB-231 cells, Dic-EFc and Ace-EFc inhibited cell migration capacity in a concentration-dependent manner (p < 0.001 vs. leptin) (Figure 4A–C).

Considering the greater invasive capacity of MDA-MB-231 cells, we delved into analyzing the effect of extracts on the migration and invasion ability of these cells. A transwell assay confirmed that 5–10 μg/mL of Dic-EFc and Ace-EFc were sufficient to inhibit leptin-induced MDA-MB-231 cell migration at 24 h of treatment (Figure 5A–C).

### 3.4. Ace-EFc and Dic-EFc extracts inhibit the invasion capacity of MDA-MB-231 cells, decreasing the secretion of MMP-2 and MMP-9

*F. crocata* extracts significantly decreased the invasion promoted by leptin. Ace-EFc had a greater effect with 20 μg/mL (p < 0.001), while Dic-EFc presented this same activity with the concentration of 10 μg/mL and 20 μg/mL (p < 0.001) (Figure 6A). Moreover, Ace-EFc and Dic-EFc at all evaluated concentrations (5, 10, and 20 μg/mL) significantly decreased leptin-induced MMP-9 and MMP-2 secretion (p < 0.01) (Figure 6B and C).

### 3.5. Ace-EFc and Dic-EFc reduce mesenchymal phenotype and F-actin polymerization in MDA-MB-231 cells

MDA-MB-231 cells have fibroblastoid morphology, with F-actin fibers distributed throughout the cytoplasm, especially at the cellular edges. Leptin exposure intensified the elongated morphology of cells and promoted the formation of membrane pseudopods; however, Ace-EFc and Dic-EFc promoted the perinuclear relocation of F-actin and a spheroid...
morphology compared to leptin-treated cells, which maintained a mesenchymal phenotype (Figure 7). The above suggests that treatment with the extracts could lead tumor cells to an epithelial phenotype associated with a lower cell migration and invasion capacity.

4. Discussion

This study aimed to explore the potential of F. crocata leaf extracts as inhibitors of migration and invasion of breast cancer cells MCF7 and
MDA-MB-231, representing tumors of subtype Luminal A and TNBC, respectively. The three extracts of *F. crocata* (Hex-EFc, Dic-EFc, and Ace-EFc) showed cytotoxic activity in tumor cells in different concentration ranges. Interestingly, low concentrations of Dic-EFc and Ace-EFc showed cytotoxic activity in MDA-MB-231 cells (IC_{50} = 40.92 and 54.89 μg/mL). In contrast, higher concentrations Dic-EFc and Ace-EFc were required to induce a cytotoxic effect on non-tumor MCF-10A cells (IC_{50} = 121.55 and 276.84 μg/mL, respectively). Previously, we reported that Dic-EFc and Ace-EFc induced apoptosis in MDA-MB-231 cells, increasing the expression of p53, procaspase-8, and procaspase-3, and cell cycle arrest (Anchez-Valdeolivar et al., 2020). Chen et al., 2017, reported the cytotoxic activity of *Ficus virens* extracts on MDA-MB-231 and MCF-7 cells by increasing the production of reactive oxygen species (ROS) and inducing apoptosis in tumor cells. The response to the treatment of *F. virens* was very similar in both tumor lines; however, its cytotoxic activity in non-tumor cells was not evaluated. This dual role has been observed in some antioxidant extracts, acting as cytoprotective substances in non-tumor cells and acting as powerful prooxidants in tumor cells, promoting cell death (Kalinowska et al., 2021; De la Cruz-Concepción et al., 2021).

On the other hand, MCF-7 cells showed resistance to treatment with extracts (Hex-EFc IC_{50} > 590, Dic-EFc IC_{50} > 550 μg/mL, and Ace-EFc IC_{50} > 1100 μg/mL); however, low concentrations (20–40 μg/mL) of Ace-EFc appear to decrease the proliferation capacity of these cells (Figure 2). These results indicate that *F. crocata* leaf extracts, particularly Dic-EFc and Ace-EFc, could have selective activity on MDA-MB-231 cells representative of TNBC compared to MCF-7 and MCF-10A cells. Estrogen receptors (ER) regulate the expression of genes related to cell proliferation and apoptosis in breast cancer cells (Brisken and O’Malley, 2010). There are two types of ER: ERα and ERβ; ERα positively regulates cell proliferation, while ERβ opposes the action of ERα by modulating the expression of genes regulated by ERα (Clarke, 2003). MCF-7 cells express both ERs, unlike MDA-MB-231 cells (Xiao et al., 2014); therefore, proliferation, apoptosis, and cell migration in MCF-7 could be regulated by ERα and ERβ. It has been reported that phytoestrogens increase ERβ expression and inhibit MCF-7 proliferation only at high concentrations (100 μM) (Chen et al., 2013). In contrast, at low concentrations (0.01–10 μM), natural compounds with estrogenic activity act as a ligand of ERα (Yuan et al., 2012), favoring the activation of metabolic and signaling pathways that promote cell proliferation and survival, such as IGF-IR, EGFR, p21, Ras and Raf-1, MAPK, ERK and PI3K/AKT (Skandalis et al., 2014). Dic-EFc and Ace-EFc contain phytosterols, such as stigmasterol, stigmastan-3,5-dien, and β-sitosterol (Sánchez-Valdeolivar et al., 2020; De la Cruz-Concepción et al., 2021), of which estrogenic activity has been high.
reported in MCF-7 cells (Sriraman et al., 2015); the above could explain why *F. crocata* extracts had significantly higher cytotoxic activity in MDA-MB-231 cells and even in MCF-10A compared to MCF7 cells.

Regarding cell migration, Hex-EFc promoted the migration of MCF-7 and MDA-MB-231 tumor cells at a level like or even higher than leptin, which is known to be an inducer of cell migration and invasion (Juárez-Cruz et al., 2019). These results could be related to high concentrations of lup-20(29)-en-3-ol acetate and lupeol in Hex-EFc (Sánchez-Valdeolívar et al., 2020). It has been reported that lupeol and β-sitosterol present in the hexane extract of *F. racemose* promote the migration capacity of the BHK 21 and MDCK kidney cells (Bopage et al., 2018). Another study reported that lupeol increased MMP-2 activity in human epidermal keratinocytes, promoting the capacity for cell migration and invasion (Pereira Beserra et al., 2018). Considering these observations, we excluded Hex-EFc for evaluation as a possible inhibitor of migration and invasion of breast cancer tumor cells.

Dic-EFc and Ace-EFc decreased the migration of MDA-MB-231 and MCF-7 cells, even with leptin, possibly by phytoestrogens and triterpenoids in the extracts as β-sitosterol, stigmastanol, and phytol. β-sitosterol has been reported to induce apoptosis and decrease cell migration and invasion of MDA-MB-321 cells by inhibiting the Ras/ERK and PI3K/AKT signaling pathways (Awad et al., 2007). It has been reported that stigmastanol decrease migration and blocks cell cycle progression by inhibiting the JAK/STAT signaling pathway in gastric cancer SNU-1 cells (Li et al., 2018). Moreover, phytol inhibited the phosphorylation of AKT and mTOR in gastric adenocarcinoma AGS cells, inhibiting migration and inducing apoptosis and cell cycle arrest in the G1 phase, negatively regulating Bcl-2 expression and increasing Bax expression (Song and Cho, 2015). The effect of Dic-EFc and Ace-EFc on cell motility is possibly due to a synergistic effect between the compounds present in the extracts; it has been reported that the synergy of phytochemical mixtures produces a better response than individual compounds (Liu, 2004).

On the other hand, we observed that Dic-EFc and Ace-EFc inhibited the invasive capacity of MDA-MB-231 cells, decreasing the secretion of MMPs. MMPs are enzymes responsible for degrading the basement membrane and extracellular matrix constituents, which are indispensable for the metastatic spread of tumor cells (Turunen et al., 2017). These observations are consistent with other species of the genus *Ficus*; in SiHa and HeLa cells, the aqueous extract of *Ficus religiosa* at concentrations of 40 and 80 μg/mL decreased MMP-2 secretion after 24 h of treatment (Choudhari et al., 2013). In addition, it has been reported that ethanol extract of *Ficus septica* reduced blood vessel formation and MMP-9 expression in a chorioallantoic membrane model (Hamid, 2021).

Finally, treatment with Dic-EFc and Ace-EFc in the MDA-MB-231 cells caused a perinuclear redistribution of F-actin, decreased the size of the cells, and caused changes in cell morphology, bringing the cells to a more circular shape. The morphological modification of MDA-MB-231 cells suggests that Dic-EFc and Ace-EFc could reverse the Epithelial-Mesenchymal Transition (EMT) process and, consequently, decrease the migration invasion capacity of the cells; however, this hypothesis still needs to be evaluated. Preliminarily, we observed by fluorescence microscopy that Dic-EFc and Ace-EFc decreased the expression levels of vimentin and increased the expression of E-cadherin (data not shown). Vimentin is a protein considered an essential regulator of EMT overexpressed in several tumor cell lines (Kidd et al., 2014). On the other hand, E-cadherin is a fundamental molecule for cell adhesion that functions as a suppressor of invasion by maintaining cell-cell junctions; loss of E-cadherin expression has been associated with EMT (Petrova et al., 2016). The decrease in mesenchymal phenotype markers (vimentin) and the increase in the expression of epithelial phenotype markers (E-cadherin) could explain the decreased migration and invasion capacity of MDA-MB-231 cells. In other studies, pantothenic acid, isolated from *F. microcarpa*, reversed the EMT process by favoring the expression of epithelial markers (E-cadherin, ZO1, and laminin) and decreasing the expression of mesenchymal markers (N-cadherin, vimentin, and Rac-1) in prostate cancer cell lines DU145, PC3, NA22 and NB26 (Akhtar et al., 2018).

In conclusion, the leaf extracts of *F. crocata* (Dic-EFc and Ace-EFc) at low concentrations (5–20 μg/mL) decreased the proliferation, migration, and invasion capacity of TNBC MDA-MB-231 cells at non-cytotoxic concentrations for non-tumor MCF-10A cells. MCF-7 breast cancer cells showed more resistance to the extracts in cell proliferation, except for Ace-EFc; however, Dic-EFc and Ace-EFc at low concentrations also decreased the migration ability of these cells. The above supports the potential use of compounds from leaf extracts of *F. crocata* as an alternative or complementary neoadjuvant therapy to conventional drugs to reduce the tumor size and prevent breast cancer progression, mainly triple-negative tumors.

Declarations

**Author contribution statement**

Lorena Cayetano-Salazar; Brenda de la Cruz-Concepción: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
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Data included in article/suppl. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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