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Feijoa sellowiana: A Natural Extract with Cytotoxic Effects on Breast Cancer Cells

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ABSTRACT
Breast cancer remains a leading cause of mortality among women, necessitating heightened attention and innovative treatment approaches. Given the heterogeneous nature of breast cancer, exploring novel therapeutic avenues is crucial. Natural products, with their potential to offer less aggressive alternatives to conventional chemotherapy, have garnered interest. In this study, the potential cytotoxic effect of *Feijoa sellowiana* fruit extract (FE) was investigated on a panel of human breast cancer cells. GC-MS analysis was performed to identify the active constituents present in the FE extract and MTT analysis was conducted to evaluate the cytotoxicity of FE against breast cancer cells. Results showed a strong efficacy of FE against MDA-MB-453 and MDA-MB-231 cell lines. The cytotoxicity was evident after a 24-hour treatment duration for both lines. It was observed that the two cell lines in which the FE extract was most effective belonged to the triple-negative breast cancer category. The viability of MCF-7 cells decreased to 23.2% after 72 hours of exposure to 1000 µg/mL FE, and this decline was also noticeable at lower concentrations. Conversely, the BT-474 cell line displayed the least susceptibility, with a viability of 43.9% even at the highest concentration of 1000 µg/mL FE. These findings underscore FE's targeted efficacy against triple-negative breast cancer cells, indicating its promise as an alternative avenue to tackle this formidable cancer subtype.

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Introduction

Cancer occurrence and cancer-related death rates over the world continue to increase. Among women, breast cancer holds the top position in terms of cancer diagnoses with 2.26 million cases according to GLOBOCAN cancer statistics in 2020 [1]. It was also shown that breast cancer-related death rates were increasing over time. Reoccurrence rates were also shown at relatively high rates [2, 3].
Breast cancer is a heterogeneous disease from both genetic and clinical aspects [4]. There are different classification approaches to breast cancer subtypes. These classifications can be made based on the origin or their receptor types [5-9]. One of these classification strategies uses the hormone receptors of estrogen receptor, ER (+/-), progesterone receptor, PR (+/-) along with the human epidermal growth factor receptor2, HER2 (+/-) [10]. Thus, the treatment approaches also change based on these molecular subtypes of breast cancer. Hormone therapies can be used against hormone receptor-positive breast cancers. Nevertheless, the primary treatment approach for triple-negative breast cancer remains aggressive chemotherapy. As a result, it is crucial to find different treatment options for more effective treatment approaches.
*Feijoa sellowiana (*O. Berg*)* O. Berg *(*synonym, *Acca sellowiana)* [11], represents a small tree displaying distinct cultivars, commonly identified as pineapple guava. It belongs to the Myrtaceae family. Feijoa, originally indigenous to South America, has experienced both natural and commercial cultivation in various countries, including Brazil, Argentina, Chile, Colombia, Uruguay, and New Zealand. This tree bears a resemblance to the olive tree in terms of its appearance and thrives under comparable environmental conditions and growth patterns. Feijoa was introduced to Turkey in 1989, subsequent to which dedicated adaptation orchards were established in Sakarya, Antalya, Mersin, and İzmir [12, 13].
The feijoa fruit has a distinctive aromatic flavor. Due to the rich bioactive compounds of feijoa, it has significant pharmacological potential. As demonstrated by various studies, feijoa fruit extract and its essential oil exhibit notable antimicrobial properties [14-16]. It was also shown that the feijoa plant has antioxidant,

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anti-inflammatory, immune-stimulating and anti-cancer activities [12, 17, 18]. Several studies reported that the feijoa extracts are effective tools for anti-cancer activities as well as to fight against multidrug resistance [19-21]. However, screening of the cytotoxic activity of the feijoa extract has not been examined in detail in different breast cancer cell lines with different properties.

This study aimed to provide a screening of the effect of feijoa fruit extract (FE) on different breast cancer cell types. Along with the volatile compound composition of FE, the potential relationship between the breast cancer types and the chemical compounds was observed. Thus, this study could provide a basis for further research on latent alternative treatment approaches for different breast cancer types.

Material and Methods

Collection and Extraction of Feijoa fruit

Feijoa fruit was collected from a local garden in Özdere-İzmir/Turkey at the end of the October since its harvest time is in the second week of October and ended in the last week of November [13]. The whole fruit including the fruit peel and seeds was used for extraction. First, 10 gr fruit was mixed in 50 mL absolute ethanol at room temperature using a homogenizer. Then the solution was ultrasonically extracted for 30 minutes. After waiting for cooling down to room temperature, it was stored at 4ºC. Before use, 0.45µm sterile filters were used to filter the feijoa fruit extract [22].

Cell culture conditions

Four distinct breast cancer cell lines were employed to assess the cytotoxicity of FE. These cell lines were MCF-7 (mammary gland adenocarcinoma, ATCC HTB-22), MDA-MB-231, mammary gland adenocarcinoma, ATCC HTB-26), BT-474, mammary gland ductal carcinoma, ATCC HTB-20) and MDA-MB-453, mammary gland carcinoma, ATCC HTB-131). All four cell lines were cultured in RPMI-1640 (Roswell Park Memorial Institute medium 1640) with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin-streptomycin [23]. Cells were maintained in 75 cm^2 polystyrene filtered cap flasks (Corning Life Sciences, UK) in a 37°C incubator with 5% CO_2. Cell growth rates, confluence, and morphologies were observed daily with an inverted microscope (Zeiss Primovert, Germany).

Cell viability assay

The cytotoxic effects of FE were assessed using the MTT assay, which measures metabolic activity through a colorimetric approach. For each cell line, a seeding density of $5x10^4$ cells per well in 96-well plates was maintained during the 24, 48, and 72-hour experimental time points. Cell counts were determined by staining with trypan blue and employing an automated cell counter (Countess, Invitrogen). Following cell seeding, plates were incubated in a CO_2 incubator for 24 hours, after which varying concentrations of FE were introduced into the wells. All the concentrations were added as triplets. After the incubation period of the plates, MTT was added to each plate as 10% of the final volume. Plates were further incubated for 4h after MTT treatment. Upon completion of the incubation period, the culture medium was aspirated, and DMSO (Sigma-Aldrich) was introduced into each well [24]. Absorbance readings at 570 nm, with a reference wavelength set at 690 nm, were recorded using a microplate reader. (Tecan Infinite M200 PRO, Switzerland).

Gas chromatography-mass spectrometry (GC-MS) analysis

For GC-MS analysis of FE a gas chromatography system (Agilent Technologies 7890A) with a mass spectrometer (5975 C mass spectrometer) was used in electron-ionization (EI) mode as described in previous studies [25, 26]. To begin with, FE ethanol extract was centrifuged at 15,000 rpm, the pellet was removed, and the supernatant of the extract was transferred to the vial for GC-MS analysis. Agilent HP-5 MS capillary column with 0.25 µm film thickness was used as the chromatographic column. Helium was used as a carrier gas at a flow rate of 1 mL min^{-1}. The database of the National Institute of Standard and Technology (NIST) was used as the source database to comment on the results.

Statistical analysis

The statistical analyses of the MTT assay results were performed with Graph Pad Prism 5 (USA) [27, 28]. The one-way ANOVA (Dunnett's t-test) was used for data analysis. Values with $p \leq 0.05$ (*) were considered statistically significant. Biosoft CalcuSyn 2.1 (USA) was used to calculate the IC_{50} values for each treatment [29].

Results and Discussion

Plant extracts are widely recognized for their bioactivities, which stem from their remarkable selectivity and, in many cases, their ability to biodegrade into non-toxic substances. This enables their application like conventional chemical drugs but in a less harmful manner. Numerous studies have documented the significant biological characteristics of Feijoa fruit extracts, highlighting their potent antimicrobial properties and remarkable antioxidant activity [14, 30]. It is also shown to have strong anticancer properties and display

tumor-selective activity in several studies [14, 21, 31-33]. Some studies also show the anti-cancer effect of feijoa-derived silver nanoparticles on MCF-7 [21, 34].

Nevertheless, no research has been conducted to study and compare the cytotoxic impact of FE on various breast cancer cells. Due to the distinct nature of various types of breast cancers, different treatment approaches are necessary to address their responses. Therefore, this study established a framework to comprehend the diverse effects of FE on distinct types of breast cancer cells.

First, the cell viability was determined. For this aim, MCF-7, MDA-MB-231, MDA-MB-453, and BT-474 breast cancer cell lines were treated with increasing concentrations (50-1000 µg/mL) of FE for 24h, 48h, and 72h. Then MTT assay was performed. As shown in Figure 1.a, the highest decrease in the cell viability of MCF-7 cells was observed in 72h as compared to the untreated control. The cell viability for 1000 µg/mL was 67.4%, 53.1%, and 23.2% in 24, 48, and 72h, respectively. The IC_{50} value of the MCF-7 at 72h was 139.3 µg/mL (Figure 2). The viability of the MDA-MB-231 cells was decreased starting from the 24h treatment and the IC_{50} value was 121.1 µg/mL at 72h (Figure 1.b, Figure 2). For the 1000 µg/mL FE, the cell viability was measured as 16% even at the end of the 24h.

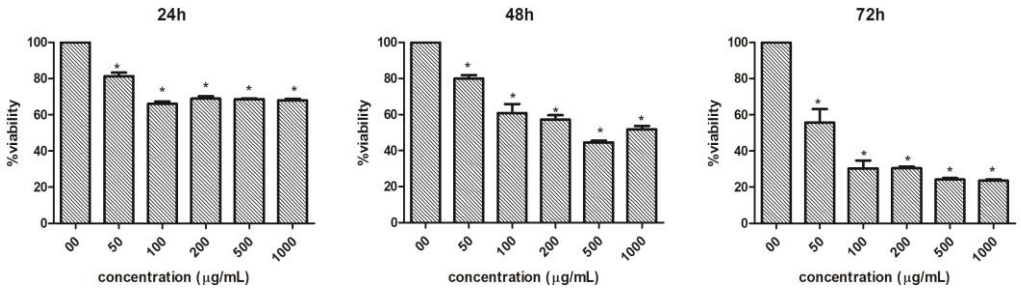
The least effect of the FE on the cell viability was observed on BT-474 (Figure 1.c). Although the cell viability was 43.9% for the 1000 µg/mL FE, the overall effect was lower as compared to the other breast cancer cell lines. Conversely, the effect of FE was already significant for 200 µg/mL in all 24, 48, and 72h treatments of MDA-MB-453. The viability was measured as 31.7%, 26.3%, and 25.2% and the IC_{50} values were 277.4, 286.0, and 121.1 µg/mL respectively (Figure 1.d, Figure 2).

The volatile compound composition was identified by GC-MS analysis of the whole fruit extract. GC-MS analysis results were listed in Table 2 including the molecular formula and NIST scores. The obtained results were compared with the previous studies on feijoa.

Overall, the results showed that the FE was highly effective on MDA-MB-453 and MDA-MB-231 cell lines. Its effect was apparent starting from the 24h treatment for both cell lines. This similarity draws attention that both cell lines are triple-negative breast cancer cell lines. So, it shows that FE holds great potential as an alternative to aggressive chemotherapy treatment. However, FE was not only effective on MDA-MB-453 and MDA-MB-231 cell lines. It has a time-increasing level of cytotoxicity for the MCF-7 cell line as well.

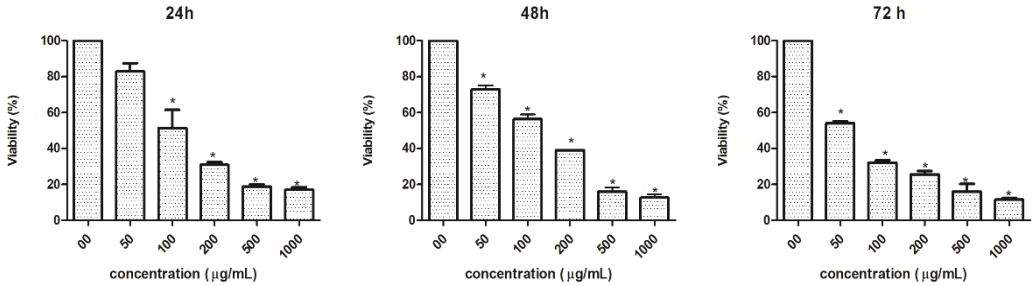
It was observed that the cell viability of the MCF-7 cell line at 72h was dropped to 23.2% with 1000 µg/mL FE but it decreased the viability even at the lower concentrations. It was also shown in another study with Crystal violet assay that, feijoa extract showed an anti-proliferative effect on MCF-7 and MDA-MB-231 cell lines. However, cell cycle analysis between these two cell lines was different and the effect on MCF-7 cells was in S or G2/M phases [31]. Other studies showed the effect of feijoa extract as green-synthesized nanoparticles on MCF-7 cells as well [21, 34].

MCF-7



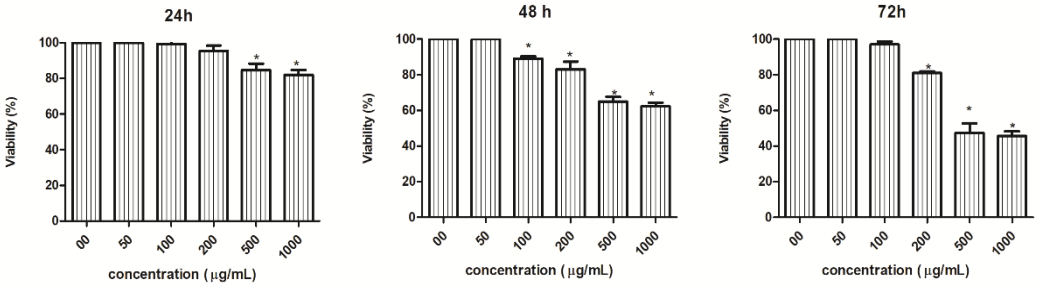
1.a

MDA-MB-231



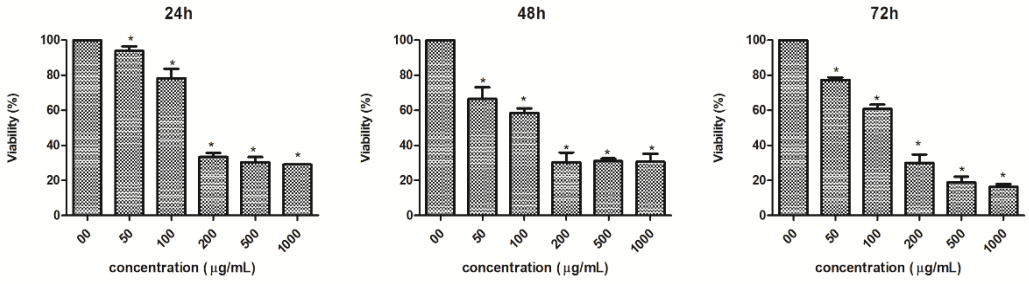
1.b

BT-474



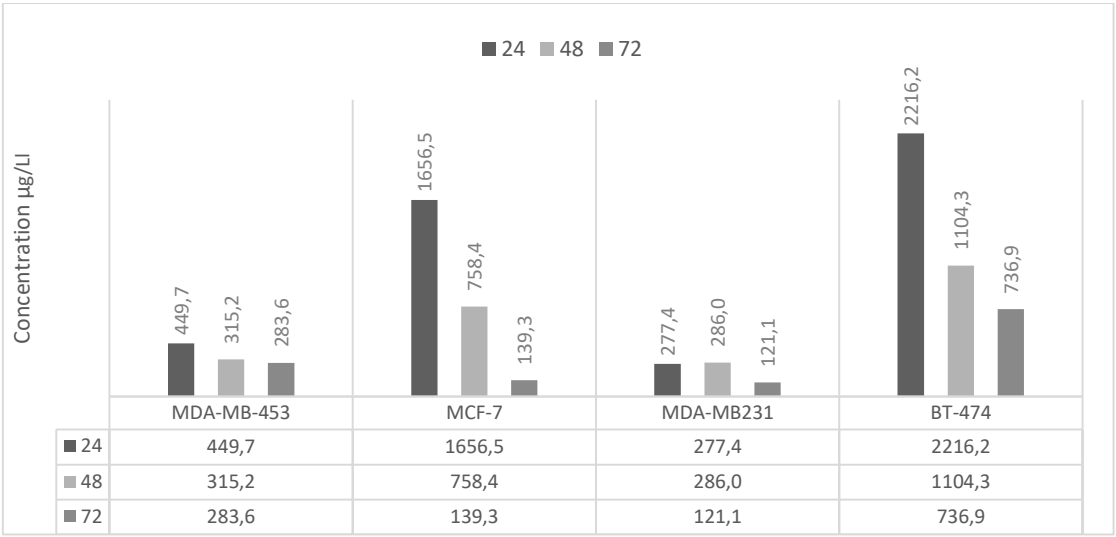
1.c

MDA-MB-453



1.d

Fig 1 Time and concentration-dependent effect of FE extract on the viability of 1.a, MCF-7; 1.b, MDA-MB-231; 1.c, BT-474; and 1.d, MDA-MB-453 cells (*P < 0.05 compared to untreated control)



	MDA-MB-453	MCF-7	MDA-MB231	BT-474
24	449,7	1656,5	277,4	2216,2
48	315,2	758,4	286,0	1104,3
72	283,6	139,3	121,1	736,9

Fig 2 IC_{50} results in µg/mL for the four different breast cancer cell lines in 24, 48, and 72 h.

The least effect on cell viability was shown against the BT-474 cell line. Then again, the cell viability was 43.9% for the 1000 µg/mL FE. The main difference was while the cytotoxicity of FE was observable even at the lower doses and/or early treatment times, it was only evident for the high doses and 72h treatment for the BT-474 cell line.

It is important to understand the relation between the cytotoxic effect of the FE and the breast cancer cell lines. Therefore, as seen in earlier research, the use of GC-MS analysis served as a foundation for establishing this connection [25, 26, 35]. For instance, there are furane derivates found in FE. Furan derivatives, known for their antibacterial, antifungal, anti-inflammatory, and anticancer properties, are currently under development as potential treatments for drug-resistant infections [36, 37].Eugenol, with its potential uses in cancer and Alzheimer's treatment, serves as an antiseptic against bacteria, fungi, and viruses, offers local anesthesia as an analgesic, reduces inflammation for conditions like arthritis, and provides antioxidant protection to cells. It's also under investigation for antimicrobial applications in infection treatment [38, 39]. While methyl benzoate is known for its contribution to the fruity aroma, 9-octadecenamide, and its derivatives are being investigated for a variety of potential applications including sleep-inducing and skin-care products [39, 40]. In the context of previous investigations into feijoa, the observed results align consistently with the potential utility of these compounds.

Also in other studies, it was shown that the active compound found in feijoa was flavone. Flavonoids are known to exhibit a broad range of anticancer effects. These impacts include the regulation of ROS-scavenging enzyme functions, cell cycle, prompting apoptosis and autophagy, and controlling the growth and invasiveness of cancer cells. The flavonoids have a twofold function in preserving ROS balance. In typical circumstances, they act as antioxidants, but in cancer cells, they demonstrate robust pro-oxidant characteristics, initiating apoptotic processes and diminishing pro-inflammatory signaling pathways [41]. Also, flavonoids induce excessive and prolonged autophagy. This suggests that flavonoids hold promise for treating individuals with cancer that are resistant to apoptosis [42]. As an active compound of FE, flavone is known to induce apoptosis through caspase, p16, p21, and TRAIL [31].

The responsiveness to TRAIL-induced apoptosis was influenced by the molecular phenotype of breast cancer. It was demonstrated that TRAIL exhibits a preferential ability to induce cell death in triple-negative cells [43]. Considering this information, it was consistent to observe that FE demonstrated greater efficacy against the MDA-MB-453 and MDA-MB-231 cell lines. TRAIL-mediated apoptosis is ineffective in inducing cell death in MCF7 cells, indicating their resistance to this mechanism. BT-474 cells have likewise exhibited resistance to TRAIL [43-45]. This information explains the increased impact of FE on MDA-MB-231 and MDA-MB-453 cells while demonstrating a lowered effect on MCF-7 and BT-474 cell lines. However, despite a lesser impact compared to triple-negative cell lines, a noticeable effect was still observed, particularly during the 72-hour treatment. This suggests that other active compounds present in the extract also play a significant role in breast cancer.

Table 1 GC-MS results of the FE extract. RT, Retention time. National Institute Standard and Technology (NIST) (Wiley7Nist05.L) was used as a database

Compound Name	**Molecular Formula**	**Score (NIST, similarity %)**	**RT**
2(5H)-Furanone	C4H4O2	90.33	8.879
Cyclohexanone	C6H10O	84.94	9.038
1,2-Cyclopentanedione	C5H6O2	96.58	9.158
2,5-Furandione	C5H4O3	96.78	9.683
2-Furancarboxaldehyde, 5-methyl-	C6H6O2	94.36	10.166
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one; 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone #	C6H8O4	95.96	10.698
Butanedial; Succinaldehyde; Succindialdehyde; Succinic aldehyde; Succinic dialdehyde;	C4H6O2	84.84	10.898
Benzenemethanol	C7H8O	93.63	12.109
1,3-Cyclopentanedione, 2,2-dimethyl-	C7H10O2	75.96	12.67
Pentanoic acid, 4-oxo-	C5H8O3	71.56	12.751
2,5-Furandicarboxaldehyde; 2,5-Furandicarbaldehyde	C6H4O3	88.77	13.596
2-Furancarboxylic acid, methyl ester	C6H6O3	96.73	13.768
2,3-Dihydro-5-hydroxy-6-methyl-4H-pyran-4-one	C6H8O3	81.16	13.853
Pentanal	C5H10O	82.3	13.86
1,2-Butanediol, 3,3-dimethyl-; 3,3-Dimethyl-1,2-butanediol	C6H14O2	81.23	14.193
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4	81.03	15.298
(+/-)bis(2-aminobut-3-enyl) disulphide	C8H16N2S2	84.24	15.597
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	C6H8O4	91.93	15.858
1-cyano-5-benzoyloxy-.beta.-D-ribofuranose	C13H13NO5	91.93	16.47
Dianhydromannitol	C6H10O4	89.14	17.18
2(3H)-Furanone, dihydro-4-hydroxy-	C4H6O3	59.56	17.702
D-Erythro-Pentose, 2-deoxy-	C5H10O4	80.16	17.987
2-Bromo-4,4-Di-N-Propylcyclobutanone; Cyclobutanone, 4-bromo-2,2-dipropyl-	C10H17OBr	77.63	18.66
(bistrifluoromethylamino-oxy)cyclopentane	C7H9NOF6	86.01	18.891
Soleron; 2(3H)-Furanone, 5-acetyldihydro-; .delta.-oxo-.gamma.-hexalactone; solerone	C6H8O3	82.9	18.926
1,5-dibromo-2,4-dimethyl-pentane; Pentane, 1,5-dibromo-2,4-dimethyl-	C7H14Br2	77.4	19.128
3-Hexyn-2-ol, 5-methyl-	C7H12O	70.6	19.157
3-Hydroxy-2,5,5-trimethylcyclopentanone	C8H12D2O2	65.17	19.183
Ketone, methyl 2-methyl-1,3-oxothiolan-2-yl	C6H10O2S	82.63	19.383
Methyl 1-Methyl-d3-2-propenyl Ether	C5H7D3O	69.71	20.113
Methyl 11-Acetoxy-5-methyl-4-oxoundecanoate	C15H26O5	61.62	21.09
Eugenol	C10H12O2	90.4	21.181
Methyl benzoate	C6H5CO2CH5	98.7	22.04
1,2(S)-Epoxyheptane	C7H14O	67.99	22.935
Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	C15H22	88.05	25.737
3-Pentanone	C5H10O	76.06	25.874
Dodecyl acrylate	C15H28O2	95.55	29.529
Butylphosphonic acid, dihexyl ester	C16H35O3P	50.11	30.877
n-Hexadecanoic acid	C16H32O2	89.15	35.008
5,5'-Oxy-Dimethylene-Bis(2-Furaldehyde); 2-Furancarboxaldehyde, 5,5'-[oxybis(methylene)]bis-	C12H10O5	85.54	35.631
Oleic Acid	C18H34O2	93.89	38.024
4H-1-Benzopyran-4-one, 2-phenyl-	C15H10O2	96.13	39.775
9-Octadecenamide, (Z)-	C18H35NO	78.96	42.122
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester; Palmitin, 2-mono-; Palmitic acid .beta.-monoglyceride; 2-Hexadecanoyl glycerol; 2-Monopalmitin; 2-Monopalmitoyl-sn-glycerol; 1,2,3-Propanetriol 2-hexandecanoyl ester; Glycerol .beta.-palmitate; 2-	C19H38O4	88.5	44.441
Octadecanoic acid, 2,3-dihydroxypropyl ester	C21H42O4	90.53	47.521
13-Docosenamide, (Z)-	C22H43NO	82.68	48.433

Conclusion

Feijoa fruit extracts (FE) exhibit significant cytotoxic effects on breast cancer cell lines, with the highest efficacy observed in triple-negative cells, MDA-MB-453 and MDA-MB-231. FE also demonstrates potential by showing time-dependent cytotoxicity against hormone receptor-positive MCF-7 cells. While the effect on BT-474 cells is less pronounced, the study highlights the diverse potential of FE in breast cancer treatment. Chemical GC-MS analysis reveals the presence of compounds like furan derivatives, eugenol, and flavone, all with known anticancer properties. The objective was to establish a foundational framework for future investigations into alternative latent treatments for various breast cancer subtypes. This study underscores FE's promise as a novel therapeutic agent for breast cancer treatment, warranting further investigation.

Abbreviations
FE: *Feijoa sellowiana* fruit extract; DMSO: Dimethyl sulfoxide; GC-MS: Gas chromatography-mass spectrometry; EI: electron-ionization; NIST: National Institute of Standard and Technology; RT: Retention time; ROS: Reactive oxygen species; TRAIL: TNF-Related Apoptosis Inducing Ligand; TNF: Tumor necrosis factor.

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The author confirms that the data supporting this study are cited in the article.

Compliance with ethical standards
Conflict of interest
The authors declare no conflict of interest.

Ethical standards
The study is proper with ethical standards.
Authors' contributions
All authors contributed equally to the study.

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