

## Phytochemical and Cytotoxic Properties of Gum and Leaf Extracts of *Ferula assafoetida*

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### Abstract

**Background & Objectives:** Breast cancer, a significant threat to women's health, is associated with a high annual mortality rate. Oxidative stress, characterized by the accumulation of reactive oxygen species, plays a pivotal role in cancer development. *Ferula assafoetida*, a traditional medicinal plant, has been widely used in cancer treatment. This study aims to compare the cytotoxic effects, phytochemical profiles, and antioxidant activities of *Ferula assafoetida* gum (FAGE) and leaf (FALE) extracts.

**Materials & Methods:** The herbal extracts from FAGE and FALE were prepared using the maceration method in 70% ethanol. The phenolic content and radical scavenging activity of the extracts were assessed using the Folin-Ciocalteu and diphenylpicrylhydrazyl (DPPH) assays, respectively. The cytotoxic effects of the extracts were examined on the MDA-MB-231 and MCF-7 cell lines through the MTT assay. Additionally, the morphological changes of the cell lines were observed using an inverted microscope.

**Results:** The phenolic content in FAGE ( $142.25 \pm 19.35$   $\mu\text{gGAE/mg}$ ) was significantly higher ( $P=0.01$ ) than that in FALE ( $48.15 \pm 3.01$   $\mu\text{gGAE/mg}$ ). Additionally, the radical scavenging activity and cytotoxic effects of FAGE were also significantly stronger than those of FALE ( $P < 0.05$ ). Regarding the cytotoxic effects of the extracts, FAGE exhibited  $\text{IC}_{50}$  values of  $80.59 \pm 10.98$  and  $59.39 \pm 15.89$   $\mu\text{g/mL}$  for MCF-7 and MDA-MB-231 cells, respectively, after 72 hours of incubation.

**Conclusions:** FAGE exhibited a greater cytotoxic effect on the MDA-MB-231 cell line than on the MCF-7, possibly following an estrogen-independent pattern. It appears that FAGE holds significant potential in refining oxidative stress, thereby inhibiting cell proliferation.

**Keywords:** Phenol, Flavonoid, Oxidative stress, Cytotoxicity, Cancer

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### Introduction

Mutations in critical molecules, such as proteins and DNA, drive cancer development. These alterations disrupt the normal proliferation and growth patterns of cells, leading to uncontrolled cell division and cancer formation. The antioxidant system plays a pivotal role in cancer

prevention. Induction of oxidative stress and accumulation of reactive oxygen species (ROS) in the microenvironment of normal cells are crucial factors in cancer initiation. Dysfunction of the antioxidant system can exacerbate the risk of cancer by disrupting physiological balance (1).

Breast cancer arises from the uncontrolled proliferation of cancerous epithelial cells. According to the latest World Health Organization (WHO) data, breast cancer is the most prevalent cancer

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among women, accounting for approximately 25% of all cancer cases globally. In 2020, an estimated 2.3 million new cases of breast cancer were diagnosed worldwide (2). In Iran, breast cancer is the most common cancer among women, with an estimated 11,000 new cases annually. The incidence rate of breast cancer in Iran is also higher than the global average, with an age-standardized rate of 32.1 per 100,000 women compared to the global rate of 25.4 per 100,000 women (3). Consequently, effective treatment of breast cancer is a fundamental priority for healthcare systems, particularly in Iran.

Conventional breast cancer treatments often have various clinical side effects, including therapeutic resistance, damage to healthy organs, cancer recurrence, and high costs, especially for triple-negative tumors. Triple-negative tumors lack expression of the human epidermal growth factor receptor (HER2), the progesterone receptor, and the estrogen receptor on their cell surfaces. These tumors are highly resistant to conventional treatments and can lead to the most aggressive form of breast cancer (4). Therefore, the development of effective herbal drugs with fewer side effects has become a priority for researchers in recent years (5). Certain natural compounds have demonstrated promising anti-cancer properties in laboratory studies and animal models.

Secondary metabolites in plants possess various pharmacological and biochemical properties, including antioxidant and cytotoxic activities (6). Phenols and flavonoids are two major groups of secondary compounds with well-established antioxidant and cytotoxic effects. *Ferula assafoetida* is a rich source of secondary metabolites, including phenols and flavonoids. Pharmacological studies have demonstrated its antioxidant and cytotoxic properties (7). The use of *Ferula assafoetida* leaves and gum is widespread among the general public. While some studies have explored the potential anti-cancer effects of certain compounds found in *Ferula assafoetida*, the evidence remains limited and inconclusive. The present

study aimed to compare the cytotoxic effects, phytochemical profiles, and antioxidant activities of *Ferula assafoetida* leaf and gum extracts.

## **Materials and Methods**

### **Herbal extraction**

Following identification by experts at the Fasa Medicinal Plants Research Center (FMPRC), *Ferula assafoetida* gum and leaf were assigned voucher numbers FMPRC-100-18 and FMPRC-100-19, respectively. The herbal extraction was performed using the maceration method (8). Dried hydroethanolic extracts (500 mg) were dissolved in dimethyl sulfoxide (5 mL). Sterilization of the stock solutions (100 mg/mL) of *Ferula assafoetida* gum extract (FAGE) and leaf extract (FALE) was accomplished using a 0.22  $\mu$ m filter. Preparation of a working solution (1 mg/mL) for cell culture and phytochemical evaluations involved 1:100 (v/v) dilution of the sterilized solutions with DMEM and distilled water, respectively (9). Ethical approval for the study was obtained from the Medical Ethics Committee of Fasa University of Medical Sciences (Code: IR.FUMS.REC.1400.166).

### **The Phenolic content assays**

The phenolic content of FAGE and FALE was assessed using the Folin-Ciocalteu assay. Specifically, 500  $\mu$ L of Folin-Ciocalteu reagent (10% v/v) was added to 100  $\mu$ L of the extracts at a concentration of 1 mg/mL, followed by an incubation period of 5 minutes at room temperature in the dark. Subsequently, 400  $\mu$ L of sodium carbonate (7.5% w/v) was introduced to the samples, and the resulting solutions were left at room temperature in darkness for 60 minutes. The absorbance of the samples was measured at 765 nm using a Synergy HTX multi-mode reader. Gallic acid served as the standard, and the phenolic content of the extracts was expressed in micrograms of Gallic Acid Equivalent (GAE) per milligram ( $\mu$ gGAE/mg) (10, 11). The results were

presented as mean  $\pm$  SD, and all measurements were conducted three times in duplicate.

### The power of radical scavenging

In the DPPH clearance assay, the total radical scavenging capacity of the extracts is assessed. The DPPH radical initially possesses a purple color, which transforms to yellow upon reduction by antioxidants. The loss of color indicates the antioxidant activity of the extracts. Various concentrations of the extracts (50, 100, 200, 500, and 1000  $\mu\text{g/mL}$ ) were prepared using 70% ethanol. These solutions (40  $\mu\text{L}$ ) were combined with 160  $\mu\text{L}$  of DPPH radicals (0.3 mM), thoroughly mixed, and then incubated for 30 minutes at room temperature in darkness. Subsequently, the absorption of the samples was measured at 517 nm using the Synergy HTX multi-mode reader. All measurements were replicated three times in duplicate. The control group comprised 70% ethanol (v/v), which was employed for diluting the essential oils. The percentage of total radical scavenging activity was calculated using the following equation (11-13):

Antioxidant power =  $\left[ \frac{\text{Optical absorption of the control group} - \text{Optical absorption of the experimental group}}{\text{Optical absorption of the control group}} \right] \times 100$

### Cell cytotoxicity of FAGE and FALE and morphological changes

The MTT assay was employed to evaluate the cell viability of MDA-MB-231 and MCF-7 breast cancer cell lines following exposure to FAGE and FALE. Based on a previous study, 10,000 cells were seeded per well in 150  $\mu\text{L}$  of medium and incubated to form a confluent cell population (14, 15).

The cells were then treated with varying concentrations of FAGE and FALE (25-200  $\mu\text{g/mL}$ ). The MTT assay procedure followed that of a previous study (16-18). The results are reported as percentages of cell viability. The IC<sub>50</sub> values for cell viability were calculated using four-parameter logistic regression. Morphological changes were also evaluated using an inverted microscope (11).

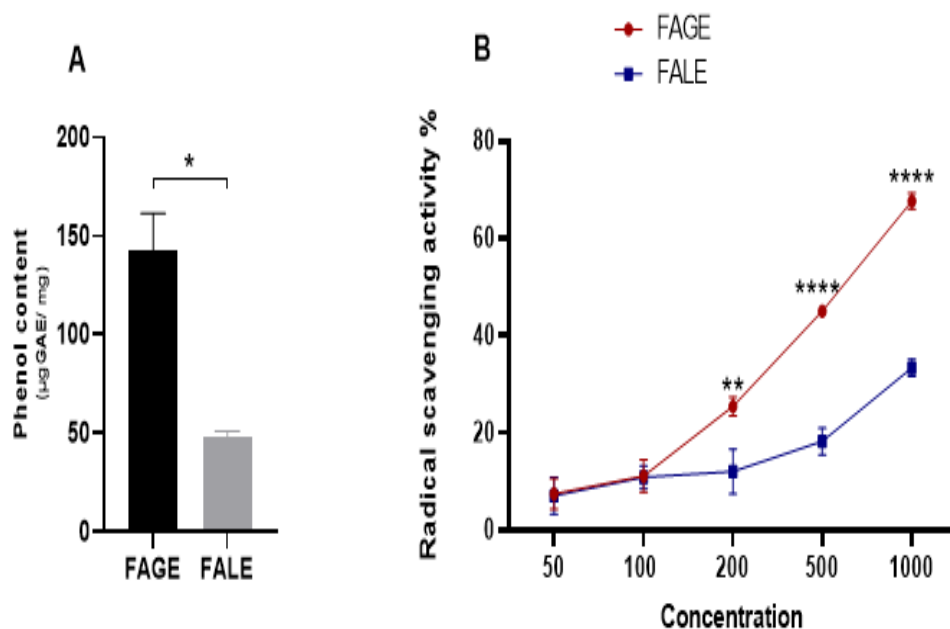
### Statistical Analysis

The data are presented as mean  $\pm$  SD. Statistical analysis was performed using the t-test in GraphPad Prism 8.0.2 software. The IC<sub>50</sub> values for cell viability were calculated using four-parameter logistic regression (19).

### Results

#### phenolic content and radical scavenging activity

According to Chart 1, the phenolic content of FAGE was significantly higher ( $P=0.01$ ) than that of FALE, with values of  $142.25 \pm 19.35$   $\mu\text{gGAE/mg}$  and  $48.15 \pm 3.01$   $\mu\text{gGAE/mg}$ , respectively. As shown in Table 1 and Chart 1, there were no statistically significant differences in radical scavenging activity between FAGE and FALE at concentrations of 50 and 100  $\mu\text{g/mL}$ . However, FAGE exhibited significantly higher radical scavenging activity than FALE at concentrations of 200  $\mu\text{g/mL}$  ( $25.46 \pm 1.99$ ,  $P=0.001$ ), 500  $\mu\text{g/mL}$  ( $45.04 \pm 0.20$ ,  $P<0.0001$ ), and 1000  $\mu\text{g/mL}$  ( $67.70 \pm 1.68$ ,  $P<0.0001$ ). Additionally, the IC<sub>50</sub> value for radical scavenging activity of FAGE (697.6  $\mu\text{g/mL}$ ) was lower than that of FALE ( $>1000$   $\mu\text{g/mL}$ ).



**Chart 1.** The phenolic content (A) and radical scavenging activity (B) of *Ferula assafoetida* gum extract (FAGE) and leaf extract (FALE)

The (\*) bullet indicates the significant differences between the group. \*, \*\*, \*\*\* and \*\*\*\* respectively indicate  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$  and  $p < 0.0001$

**Table 1.** Total radical scavenging activity of *Ferula assafoetida* gum extract (FAGE) and leaf extract (FALE) and ascorbic acid

Concentration (µg/mL)	FAGE		FALE		P-value
	Mean± SD	IC50	Mean± SD	IC50	
50	7.45± 3.13	697.6	7.03± 3.78	>1000	0.88
100	11.12± 3.37		10.84± 2.32		0.90
200	25.46± 1.99		12.02± 4.58		0.001
500	45.04± 0.20		18.24± 2.71		<0.0001
1000	67.70± 1.68		33.3± 1.82		<0.0001

### Cytotoxicity of the extracts on the MCF-7 cell line

As shown in Table 3, FAGE and FALE significantly reduced the cell viability of the MCF-7 cell line after incubation for 24, 48, and 72 hours. Following 24 hours of incubation, the IC<sub>50</sub> values of FAGE and FALE on the MCF-7

cell line were  $112.9 \pm 35.82$  and  $>200$  (more than 200)  $\mu\text{g/mL}$ , respectively. FAGE exhibited significantly higher cytotoxic effects compared to FALE at herbal extract concentrations of 50 ( $P=0.004$ ), 100, and 200  $\mu\text{g/mL}$  ( $P<0.0001$ ). The highest cytotoxic effect of FAGE was observed at the concentration of 200  $\mu\text{g/mL}$ , reaching 71.90%.

**Table 2.** Cytotoxic effects of *Ferula assafoetida* gum extract (FAGE) and *Ferula assafoetida* leaf extract (FALE)

Cell line and exposure time		Dose (µg/mL)	FAGE			FALE			P-value
			Viability %		IC <sub>50</sub>	Viability %		IC <sub>50</sub>	
			Mean	STD		Mean	STD		
MCF-7	24 h	Control	100.95	4.08	112.9 ± 35.82	100.93	1.31	> 200	0.99
		25	92.30	3.82		97.16	1.10		0.47
		50	86.55	5.03		96.73	2.00		0.004
		100	61.53	6.59		94.17	6.82		< 0.0001
		200	28.10 **	7.66		98.06	0.90		< 0.0001
	48 h	Control	100.01	4.03	98.45 ± 9.8	100.68	2.05	> 200	0.74
		25	94.36	0.36		97.26	1.66		0.36
		50	79.15	8.09		98.16	2.02		0.002
		100	48.65	6.40		87.94	5.65		< 0.0001
		200	8.95	2.20		55.98 ***	4.36		< 0.0001

	72 h	Control	99.46	1.44	$80.59 \pm 10.98$	100.14	2.69	> 200	0.57
		25	88.42	6.76		98.73	0.75		0.04
		50	66.79	6.01		97.75	2.22		< 0.0001
		100	38.82	11.67		96.66	6.56		< 0.0001
		200	5.71	2.60		82.59	7.90		< 0.0001
	24 h	Control	99.83	2.94	$173.6 \pm 39.51$	98.52	2.32	> 200	0.63
		25	88.81	3.05		97.96	2.06		0.01
		50	73.61 ***	5.99		97.50	1.11		< 0.0001
		100	55.82	5.44		95.51	4.93		< 0.0001
		200	39.47	4.71		90.12	6.89		< 0.0001
MDA-MB-231	48 h	Control	98.78	6.89	$62.76 \pm 7.32$	99.18	2.09	> 200	0.87
		25	92.92	6.52		97.51	1.66		0.30
		50	57.81 ***	6.51		97.96	0.28		< 0.0001
		100	27.18 ****	5.09		97.41	7.04		< 0.0001
		200	8.58	0.80		85.82	3.09		< 0.0001
	72 h	Control	98.03	3.41	$59.39 \pm 15.89$	99.39	0.45	> 200	0.63
		25	71.02 **	3.31		99.07	0.38		0.0001
		50	59.73	7.66		98.37	1.40		< 0.0001
		100	21.14 **	6.77		100.57	2.22		< 0.0001
		200	6.70	0.13		80.83	3.74		< 0.0001

The P-value column reports statistical differences between corresponding concentrations of FAGE and FALE through t-test analysis. The (\*) bullet also denotes statistical differences between cell lines at similar concentrations and incubation times through t-test analysis. The asterisk is placed on the cell line with lower viability % (indicating higher cytotoxic effects). \*, \*\*, \*\*\*, and \*\*\*\* respectively indicate  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ , and  $p < 0.0001$ .



After 48 hours of incubation, the IC<sub>50</sub> value for FAGE and FALE on the MCF-7 cell line was  $98.45 \pm 9.8$  and  $>200 \mu\text{g/mL}$ , respectively. FAGE exhibited higher cytotoxic effects compared to FALE at concentrations of 50 ( $P = 0.002$ ), 100, and  $200 \mu\text{g/mL}$  ( $P < 0.0001$ ). The highest cytotoxic effects of FAGE and FALE were observed at the concentration of  $200 \mu\text{g/mL}$ , reaching 91.05% and 44.02%, respectively.

After 72 hours of incubation, the IC<sub>50</sub> value for FAGE and FALE on the MCF-7 cell line was  $80.59 \pm 10.98$  and  $>200 \mu\text{g/mL}$ , respectively. FAGE demonstrated higher cytotoxic effects compared to FALE at concentrations of 50, 100, and  $200 \mu\text{g/mL}$  ( $P < 0.0001$ ). The highest cytotoxic effects of FAGE and FALE were observed at the concentration of  $200 \mu\text{g/mL}$ , reaching 94.29% and 17.41%, respectively.

### Cytotoxicity of the extracts on the MDA-MB-231 cell line

According to Table 3, both FAGE and FALE significantly reduced the cell viability of MDA-MB-231 after 24, 48, and 72 hours of incubation. After 24 hours of incubation, the IC<sub>50</sub> value for FAGE and FALE was  $173.6 \pm 39.51$  and  $>200 \mu\text{g/mL}$ , respectively. FAGE exhibited higher cytotoxic effects compared to FALE at doses of 25 ( $P = 0.01$ ), 50, 100, and  $200 \mu\text{g/mL}$  ( $P < 0.0001$ ). The highest cytotoxic effects of FAGE and FALE were observed at the concentration of  $200 \mu\text{g/mL}$ , reaching 60.53% and 9.88%, respectively.

After 48 hours of incubation, the IC<sub>50</sub> value for FAGE and FALE was  $62.76 \pm 7.32$  and  $>200 \mu\text{g/mL}$ , respectively. FAGE demonstrated higher cytotoxic effects compared to FALE at concentrations of 50, 100, and  $200 \mu\text{g/mL}$  ( $P < 0.0001$ ). The highest cytotoxic effects of FAGE and FALE were observed at concentrations of  $200 \mu\text{g/mL}$ , reaching 91.42% and 14.18%, respectively. The IC<sub>50</sub> value for FAGE and FALE

after 72 hours of incubation was  $59.39 \pm 15.89$  and  $>200 \mu\text{g/mL}$ , respectively. FAGE displayed higher cytotoxic effects compared to FALE at concentrations of 25 ( $P = 0.0001$ ), 50, 100, and  $200 \mu\text{g/mL}$  ( $P < 0.0001$ ). The highest cytotoxic effects of FAGE and FALE were observed at the concentration of  $200 \mu\text{g/mL}$ , reaching 93.3% and 19.17%, respectively.

### The cytotoxicity of the extracts between cell lines

The cytotoxic effects of FAGE on the MDA-MB-231 cell line were significantly higher ( $P = 0.0001$ ) than those on the MCF-7 cell line at the  $50 \mu\text{g/mL}$  dose after 24 hours of exposure. However, at the concentration of  $200 \mu\text{g/mL}$ , the cytotoxic effects of FAGE on the MDA-MB-231 cell line were lower than those on the MCF-7 cell line ( $P = 0.0013$ ). After 48 hours of incubation, the cytotoxicity of FAGE on the MDA-MB-231 cell line was significantly higher compared to the MCF-7 cell line at the concentrations of  $50 \mu\text{g/mL}$  ( $P = 0.0001$ ) and  $100 \mu\text{g/mL}$  ( $P < 0.0001$ ). After 72 hours of incubation, the cytotoxicity of FAGE on the MDA-MB-231 cell line was also significantly higher compared to the MCF-7 cell line at the doses of  $25 \mu\text{g/mL}$  ( $P = 0.0065$ ) and  $100 \mu\text{g/mL}$  ( $P = 0.0038$ ). Overall, the cytotoxicity of FAGE on the MDA-MB-231 cell line was higher after incubation times of 48 and 72 hours, respectively, with IC<sub>50</sub> values of  $62.76 \pm 7.32 \mu\text{g/mL}$  and  $59.39 \pm 15.89 \mu\text{g/mL}$ . At the 24-hour incubation time, the cytotoxicity of FAGE on the MCF-7 cell line (IC<sub>50</sub>:  $112.9 \pm 35.82 \mu\text{g/mL}$ ) was higher than that on the MDA-MB-231 cell line (IC<sub>50</sub>:  $173.6 \pm 39.51 \mu\text{g/mL}$ ). Regarding FALE, the cytotoxic effects on the MCF-7 cell line were significantly higher than those on the MDA-MB-231 cell line after 48 hours of incubation at the concentration of  $200 \mu\text{g/mL}$  ( $P = 0.0006$ ).

## Analysis of cell morphological changes

The morphological changes in the MCF-7 and MDA-MB-231 breast cancer cell lines exposed to FAGE and FALE exhibited a concentration- and time-dependent pattern. The morphological alterations observed in the MDA-MB-231 cell line were more pronounced than those in the MCF-7 cell line. Additionally, FAGE induced more significant morphological changes compared to FALE. The exposure to the herbal extracts led to the rupture of the cell lines, accompanied by a significant increase in cell debris and cell granules.

## Discussion

Triple-negative tumors lack the epidermal growth factor receptor 2, progesterone receptor, and estrogen receptor. This tumor type is highly resistant to common treatments and carries a poor prognosis. Ongoing efforts to discover alternative effective therapies are focusing on triple-negative cell lines such as MDA-MB-231 (20). In contrast, MCF-7 possesses the estrogen receptor (ER), making it an ER-positive tumor cell line, unlike the ER-negative MDA-MB-231. The current study revealed that FAGE and FALE exerted higher cytotoxic effects on the MDA-MB-231 triple-negative cell line compared to the MCF-7 cell line. Thus, the cytotoxic effects of FAGE and FALE appear to be independent of estrogen receptors, suggesting a potential therapeutic impact on triple-negative tumors.

The IC<sub>50</sub> values of FAGE on the MDA-MB-231 cell line were  $62.76 \pm 7.32$  and  $59.39 \pm 15.89$   $\mu\text{g/mL}$  after 48 and 72 hours of incubation, respectively. Consequently, FAGE is considered a relatively active compound according to the Baharum et al. criteria for the classification of anti-cancer herbal medicinal plants (21). The IC<sub>50</sub> values of FALE were  $>200$   $\mu\text{g/mL}$  after 48 and 72 hours of incubation, categorizing FALE as a weakly active compound. On the MCF-7 cell line, FAGE induced a higher decrease in cell viability or greater cytotoxic effects. The IC<sub>50</sub> values of FAGE were  $98.45 \pm 9.8$  and  $80.59 \pm 10.98$   $\mu\text{g/mL}$  on the MCF-7 cell line after 48 and

72 hours of incubation, respectively, confirming its relatively active nature on the MCF-7 cell line.

The heightened cytotoxic effects of FAGE are likely attributed to its higher radical scavenging activity, more potent reducing power, and greater phenolic and flavonoid contents. FAGE appears to possess a substantial capacity for improving oxidative stress, a factor in tumor progression that disrupts the microenvironment of normal cells, leading to changes in physiological parameters such as the suppression of inhibitory signals, production of growth signals, and angiogenesis. Natural antioxidants like polyphenols and flavonoids exhibit high efficacy in improving oxidative stress (22). It is plausible that the polyphenols and flavonoids in FAGE inhibit cancer progression through oxidative stress improvement, whereas FALE, with lower phenolic and flavonoid content, exhibits comparatively lower cytotoxic effects.

In contrast to the present study, Niazmand et al. reported that FALE has higher phenolic and flavonoid content and superior antioxidant effects compared to FAGE (23). This difference may be attributed to variances in the extraction method. Niazmand et al. immersed *Ferula assafoetida* leaf and gum for 3 hours in the solvent, while the present study employed a one-week incubation period. This prolonged incubation likely led to a more comprehensive extraction, resulting in the extraction of a greater number of compounds, as confirmed by the phenolic and flavonoid content results. Additionally, *Ferula assafoetida* gum exhibits different compositions depending on the time of harvest and climatic conditions, presenting in white, pink, brown, and black colors. Hence, the gum used in this study may have had diverse compositions (24).

Some studies have reported the cytotoxic and anticancer effects of isolated bioactive components from *Ferula assafoetida*. Hasanzadeh et al. documented the cytotoxic and antioxidant effects of Farnesiferol C, one of the major compounds found in *Ferula assafoetida* (36). Kim et al. reported the anti-tumor effect of isolated Galbanic



acid from *Ferula assafoetida* (37). Iranshahy et al. also reported the cytotoxic effect of Gummosin on PC-3 and MCF-7 cell lines. Gummosin, a sesquiterpene coumarin isolated from *Ferula assafoetida*, exhibited cytotoxic effects with IC<sub>50</sub> values of 32.1 and 30 µg/mL on MCF-7 and PC-3 cell lines, respectively (38).

To the best of our knowledge, this study is the first to compare the cytotoxic effects of leaf and gum extracts of *Ferula assafoetida* on breast cancer cell lines. FAGE demonstrated more potent cytotoxic effects, likely in an estrogen-independent pattern. It is recommended that future studies evaluate the efficacy of different methods of gum extraction. One of the most significant limitations of the present study was the failure to investigate the cytotoxic effects of FAGE and FALE on non-cancerous cells.

### **Conclusion**

The rising prevalence of breast cancer and the increasing resistance to conventional therapies underscore the urgent need for novel therapeutic approaches. This study demonstrates that FAGE exhibits significantly higher cytotoxic effects compared to FALE, likely due to its richer phytochemical content and enhanced antioxidant activity. Based on the classification criteria for anticancer compounds, FAGE is considered a relatively active agent. FAGE displayed a more pronounced cytotoxic effect on the MDA-MB-231 cell line compared to MCF-7, suggesting an estrogen-independent mechanism of action. Moreover, the cytotoxic effects of FAGE intensified with increasing concentration and incubation time. Therefore, FAGE emerges as a promising drug candidate for the treatment of triple-negative tumors.

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### **Conflict of Interests**

None.

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### **Ethical Considerations**

Non related.

### **Code of Ethics**

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### **Author's Contributions**

AM assumed the responsibility for conceptualizing and designing the study, conducting the MTT assays, and meticulously analyzing the gathered data. RR diligently carried out the MTT assays and executed the phytochemical analyses with utmost precision. ADA meticulously performed the phytochemical analyses, ensuring the accuracy and reliability of the results. KR provided invaluable assistance in the extraction of plant materials and the execution of phytochemical analyses. AK made significant contributions to the study design, providing expert guidance and direction. MM collaborated extensively in the preparation of the manuscript, lending their expertise to enhance the clarity and coherence of the writing. SN initiated the study and played a pivotal role in its design, providing the foundation for the research endeavor. All authors actively participated

in drafting the manuscript, meticulously crafting each section to convey the findings effectively. Finally, all authors thoroughly reviewed and approved the final manuscript, ensuring the accuracy, completeness, and overall quality of the research presentation.

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