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Experimental Research Article

The Effect of Different Polar Solvents on the Extraction of Bioactive Compounds in *Ferula* assafoetida and Subsequent Cytotoxic Effects

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ABSTRACT

Introduction: The cytotoxic effects of *Ferula assafoetida* extract intensely depend on high-quality extraction. The type of solvent used is a critical parameter for efficient extraction in the maceration method. In the present study, the phytochemical and cytotoxic effects of different *Ferula assafoetida* extracts were compared.

Methods: The *Ferula assafoetida* gum was extracted using different polar solvents: hydroethanol (70% v/v), dimethyl sulfoxide (DMSO), and water. The phytochemical properties of the extracts were evaluated, focusing on their herbal content of phenols and flavonoids. The antioxidant activity of the extracts was also compared by assessing their radical scavenging capacity (by DPPH assay) and reducing activity (using the FRAP assay). Finally, the cytotox effects of the extracts were evaluated using the MTT assay on MCF-7 and MDA-MB-231 breast cancer cell lines for the first time.

Results: The phytochemical properties of hydroethanolic extract of *Ferula assafoetida* (HEFA) were significantly ($P< 0.0001$) higher than those of the DMSO (DEFA) and water extracts (WEFA). The reducing power, radical scavenging activity, and cytotoxic effects of HEFA were also significantly ($P< 0.05$) higher than those of DEFA and WEFA. The cytotoxicity of the extracts was dose- and incubation time-dependent. HEFA exhibited the highest cell cytotoxicity at 72 hours, with IC50 values of 69.97± 9.45 µg/mL on the MCF-7 cell line and 60.22± 2.37 µg/mL on the MDA-MB-231 cell line.

Conclusion: Hydroethanol was the best solvent for extracting phenolic compounds and flavonoids. The cytotoxic effects of HEFA were also the highest, probably due to the high ability of hydroethanol in the extraction of hydrophilic and lipophilic phenols.

Keywords:
Solvent
Bioactive compounds
Oxidative stress
Cytotoxicity
Ferula assafoetida

Introduction

Breast cancer occurs when abnormal cells in the breast grow and divide uncontrollably under conditions of oxidative stress, leading to the formation of a tumor (Trayes

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et al., 2021). Oxidative stress is an imbalance between the oxidant and antioxidant systems. In the context of breast cancer, oxidative stress plays a complex role. It can contribute to the initiation and progression of breast cancer through various mechanisms such as DNA damage, activation of signaling pathways involved in cell proliferation, trigger chronic inflammation, and impairment of anticancer defense mechanisms (Jelic et al., 2021). Therefore, focusing on the antioxidant system and oxidative stress improvement is critical.

Phenolic compounds and flavonoids are therapeutic metabolites in many herbal extracts. These compounds have been studied for their ability to improve oxidative stress. They can reduce oxidative stress by multiple mechanisms such as oxidant scavenging, antioxidant enzyme activation, metal chelation, anti-inflammatory effects, and cell signaling regulation in antioxidant defense systems (Shen et al., 2022).

Phenolic compounds and flavonoids can scavenge and neutralize oxidants. They can also activate antioxidant enzymes (catalase, SOD, and glutathione peroxidase) that can break down harmful oxidants and minimize their damaging effects (Rana et al., 2022). Some phenolic compounds and flavonoids have the ability to chelate or bind to metal ions, such as iron and copper. These metal ions can participate in reactions that generate oxidants. By binding to these metal ions, phenolic compounds, and flavonoids can prevent or reduce oxidant production, thus helping to alleviate oxidative stress (Lakey-Beitia et al., 2021). The phenolic compounds and flavonoids may help mitigate the production of oxidants associated with chronic inflammation by inhibiting inflammatory mediators and pathways. They also can modulate genes and proteins associated with antioxidant defense systems, leading to improved cellular responses to oxidative stress (Maleki et al., 2019).

Ferula assafoetida is a plant native to Iran and Afghanistan. It has been used in traditional medicine for centuries and is also used as a culinary spice in some parts of the world. As for its phenolic compounds and flavonoids, studies have identified several such compounds in *Ferula assafoetida* extract, including ferulic acid, umbelliferone, quercetin, kaempferol, isoquercitrin, and rutin. These compounds possess various biological activities, such as antioxidant, anti-inflammatory, and anticancer effects (Ghaffari Sirizi et al., 2023).

The solvents mainly affect on extraction of *Ferula assafoetida* phenolic and flavonoid compounds. The choice of solvent for extraction of phenolic compounds and flavonoids depends on several factors, including the solubility of the target compounds in the solvent, the polarity of the solvent, and the potential interactions between the solvent and other components in the herbal matrix (Dent et al., 2013). Multiple solvents such as hydroethanol, dimethyl sulfoxide (DMSO), and water have been used for the extraction of herbal phenol and flavonoid in *Ferula assafoetida* gum in the previous studies (Bagheri et al., 2017; Latifi et al., 2019). Hydroethanol is a mixture of ethanol and water that is commonly used. The addition of water to ethanol can increase the extraction efficiency of certain phenolic compounds that are more polar and less soluble in pure ethanol. DMSO is a polar aprotic solvent for herbal extractions due to its excellent solvating properties and ability to dissolve a variety of organic compounds. Water is another commonly used solvent for herbal extractions, particularly for polar compounds such as phenolic compounds and flavonoids.

The present study aimed to compare the effectiveness of the polar solvents (hydroethanol, DMSO, and water) in extracting phenolic and flavonoid compounds of *Ferula assafoetida*. The antioxidant activity of different *Ferula assafoetida* extracts was also evaluated by FRAP (Ferric Reducing Antioxidant Power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays. Finally, the cytotoxic effects of the extracts were evaluated on MCF-7 and MDA-MB-231 breast cancer cell lines, for the first time.

Material and methods

The Ferula assafoetida gum extraction

The *Ferula assafoetida* gum was collected from Fasa city, Fars province, Iran. The collected gum was confirmed by the experts of FMPRC and the FMPRC-100-19 voucher number was assigned to it. Extraction was done by the maceration method. The powdered gum (10 g) was immersed in 100 mL of ethanol (70:30 v/v), DMSO, and water, respectively, for the preparation of the hydroethanolic (HEFA), dimethyl sulfoxide (DEFA), and aqueous extract (WEFA) of *Ferula assafoetida*. The extract stock solution (100 mg/mL) was prepared after one week, following the complete dissolution of the gum in the solvent.

The herbal content of phenol and Flavonoid

The herbal content of phenols and flavonoids in HEFA, DFFA, and WEFA was evaluated using the Folin-Ciocalteu and aluminum chloride methods, respectively, as described in our previous studies (Moulazadeh et al., 2021a; Moulazadeh et al., 2022). Gallic acid and quercetin were used as standards for the herbal content of phenols and flavonoids, respectively. Therefore, the herbal content of phenols and flavonoids in the extracts was reported as Gallic Acid Equivalent (GAE) and Quercetin Equivalent (QE).

The antioxidant power

The antioxidant power of HEFA, DFFA, and WEFA were evaluated by the FRAP and DPPH assay according to our previous studies (Makoolati et al., 2022; Moulazadeh et al., 2022; Ranjbar et al., 2022). The FRAP and DPPH assays respectively indicate the monovalent reducing activity and the total radical scavenging capacity of the extracts. The monovalent reducing activity of the extracts was reported in the unit of μmolFe^{2+}/g. The total radical scavenging capacity of HEFA, DFFA, and WEFA was also reported in percentage (%) and was compared with ascorbic acid as a potent antioxidant compound.

Cell cytotoxicity of HEFA, DEFA, AND WEFA

Cell viability of the MDA-MB-231 and MCF-7 breast cancer cell lines in exposure to HEFA, DFFA, and WEFA were evaluated by the MTT assay. According to the previous study, the number of seeded cells was 10,000 cells per well in the volume of 150 μL and incubated to form a confluent cell population (Moulazadeh & Kouhpayeh, 2020; Moulazadeh et al., 2021b). The cells were exposed to 25-400 μg/mL doses of HEFA, DFFA, and WEFA. The procedure of the MTT assay was according to the previous study (Moulazadeh et al., 2021c). The unit of reported results was the cell viability percentage. The IC50 value of cell viability was also calculated by four parametric logistic regressions (Moulazadeh et al., 2022).

Statistical analysis

Data are expressed as the mean± SD. The t-test was used for the analysis of data in Graph Pad Prism 8.0.2 software. The IC50 value of cell viability was also calculated by four parametric logistic regression (Chen et al., 2013).

Results

Herbal content in phenol and flavonoid

According to Figure 1, the herbal content of phenol and flavonoid in HEFA was 148.23± 26.26 μgGAE/mg and 377.59± 7.12 μgQE/mg, respectively. The herbal phenolic and flavonoid content in HEFA was significantly higher than DEFA (P=0.003 for phenol and P=0.0004 for flavonoid) and WEFA (P<0.0001) extracts. The phenolic content of DEFA (86.12±6.41 μgGAE/mg) was significantly (P=0.029) higher than WEFA (45.13± 14.46 μgGAE/mg). The flavonoid content of the DEFA (234.15±26.87 μgQE/mg) was also significantly (P=0.0008) higher than that of WEFA (105.45± 18.53 μgQE/mg).

Antioxidant activity

According to Figure 2, the reducing activity of the HEFA was 1862.44± 4.64 μmolFe2+/g, which was significantly higher than that of DEFA (p=0.0001) and WEFA (P<0.0001). The reducing activity of DEFA (1078.40± 122.69 μmolFe2+/g) was also significantly

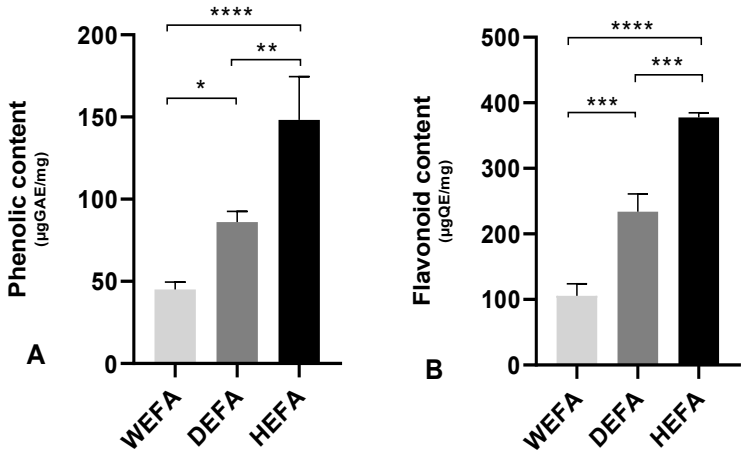

FIGURE 1. The phytochemical properties (phenolic content (A) and flavonoid content) of hydroethanolic (HEFA), dimethyl sulfoxide (DEFA), and water extract of Ferula assafoetida gum (WEFA). *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001.

TABLE 1: Phytochemical properties and antioxidant activity of hydroethanolic (HEFA), dimethyl sulfoxide (DEFA), and aqueous extract of Ferula assafoetida gum (WEFA).

	Phenol (µgGAE/mg)	Flavonoid (µgQE/mg)	Antioxidant activity	
			reducing activity (µmolFe^{2+}/g)	IC50 of radical scavenging capacity (µg/mL)
WEFA	45.13± 4.46	105.45± 18.53	618.65± 86.87	>1000
DEFA	86.12± 6.41	234.15± 26.87	1078.40± 122.69	817
HEFA	148.23± 26.26	377.59± 7.12	1862.44± 4.64	671.6
P-value	**<0.0001**	**<0.0001**	**<0.0001**	-

TABLE 2: The total antioxidant activity of hydroethanolic (HEFA), dimethyl sulfoxide (DEFA), and aqueous extract of Ferula assafoetida gum (WEFA).

CONC (µg/mL)	WEFA		DEFA		HEFA		P-value	Ascorbic acid	
	Mean± SD	IC50	Mean± SD	IC50	Mean± SD	IC50		Mean± SD	IC50
50	0.48± 0.76	>1000	0.40± 0.23	817	12.85± 4.25	671.6	**0.0102**	53.4± 3.83	48.21
100	2.12± 1.80		8.43± 1.90		14.44± 4.64		**0.0025**	72.15± 0.47	
200	3.01± 1.91		12.94± 1.68		22.13± 4.92		**<0.0001**	86.05± 0.27	
500	12.27± 2.94		35± 1.41		41.81± 4.97		**<0.0001**	89.68± 0.27	
1000	20.64± 3.49		58.77± 6.45		69.18± 2.54		**<0.0001**	89.82± 0.14	

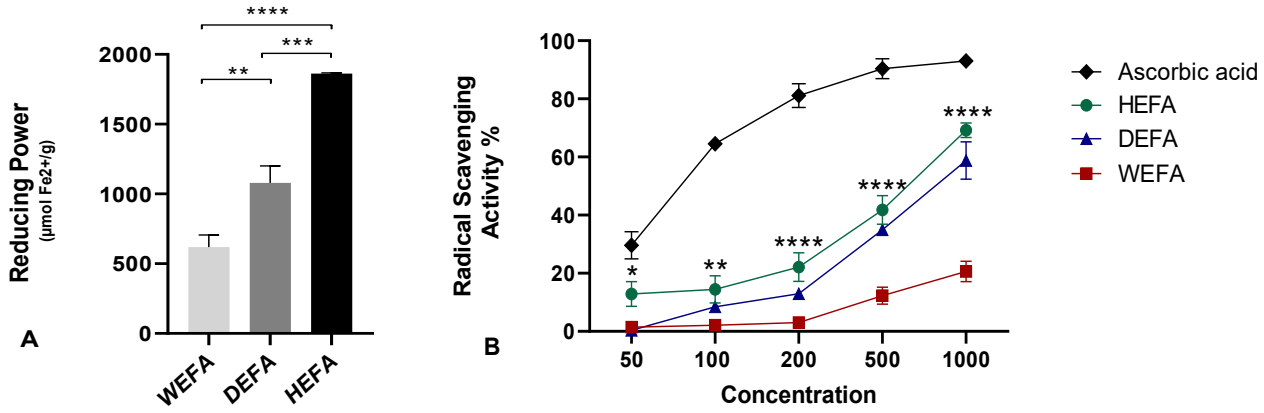

FIGURE 2. The reducing activity (A) and radical scavenging capacity (B) of hydroethanolic (HEFA), dimethyl sulfoxide (DEFA), and water extract of Ferula assafoetida gum (WEFA). *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001.

(p=0.0032) higher than that of WEFA (618.65± 86.87 µmol Fe2+/g).

About the radical scavenging capacity of the extracts, the highest capacity was related to ascorbic acid (IC50= 30.02), HEFA (IC50= 671.6), DEFA (IC50=817), and WEFA (IC50>1000), respectively. The antiradical activity of HEFA was significantly (P<0.05) higher than those of DEFA and WEFA. The antiradical activity of DEFA was significantly (P<0.05) higher than that of WEFA in 200, 500, and 1000 µg/mL doses. However, there were no significant differences in 50 and 100 µg/mL doses. The antiradical activity of HEFA, DEFA, and

TABLE 3: The cell viability of the MCF-7 and MDA-MB 231 cell lines in exposure to hydroethanolic (HEFA), dimethyl sulfoxide (DEFA), and aqueous extract of *Ferula assafoetida* gum (WEFA).

Cell line	Time	CONC (µg/mL)	WEFA			DEFA			HEFA			P-value
			Viability %		IC50	Viability %		IC50	Viability %		IC50	
			Mean	SD		Mean	SD		Mean	SD		
MCF-7	24 h	50	90.88	1.93	186.20 ± 30.81	83.76	8.71	152.40± 22.44	89.98	4.72	102± 16.91	0.35
		100	72.85	5.04		64.02	4.80		58.48	4.92		**0.04**
		200	47.55	9.26		37.36	6.68		21.39	8.46		**0.002**
		400	15.35	4.26		18.21	6.37		10.10	0.21		0.25
	48 h	50	77.51	3.05	131.30 ±16.58	65.98	6.08	100.30 ± 13.65	76.35	6.00	86.02± 15.36	0.09
		100	56.21	7.99		55.26	3.28		54.28	8.91		0.95
		200	33.77	9.93		18.43	5.17		7.96	1.15		**0.001**
		400	9.18	3.97		5.31	1.50		8.67	0.45		0.27
	72 h	50	75.44	1.62	126.30 ± 9.82	74.87	8.03	87.70 ± 10.71	72.47	12.70	69.97± 9.45	0.92
		100	60.30	6.05		39.90	7.47		38.80	15.02		**0.03**
		200	25.72	7.32		12.06	5.17		6.69	3.38		**0.001**
		400	8.77	5.25		10.88	5.21		4.98	0.35		0.49
MDA-MB-231	24 h	50	81.52	1.23	236.60 ± 37.44	79.82	1.12	137.20 ± 64.10	73.61	1.70	185.60± 75.76	**0.007**
		100	80.02	10.31		62.72	1.86		55.82	6.00		**0.04**
		200	56.83	10.35		32.40	4.66		39.47	2.72		**0.011**
		400	25.63	0.90		31.07	1.34		20.05	2.13		0.013
	48 h	50	77.48	1.26	114.40 ± 5.48	84.36	8.07	85.80 ± 4.33	60.91	5.78	61.26± 6.17	**0.023**
		100	59.25	5.32		42.69	2.71		28.00	1.31		**0.001**
		200	20.50	7.01		8.14	1.25		8.79	0.29		0.08
		400	7.70	0.29		8.04	1.10		7.79	0.74		0.09
	72 h	50	74.08	1.68	86.77 ± 4.59	81.96	4.15	83.48 ± 2.61	65.77	6.57	60.22± 2.37	0.06
		100	45.65	3.40		36.88	0.08		18.13	5.71		**0.005**
		200	16.23	1.03		8.75	0.35		7.35	0.91		**0.003**
		400	6.20	6.05		6.67	0.95		6.39	0.14		0.72

WEFA in 1000 µg/mL dose compared to 50 µg/mL increased by 56.33 %, 58.37 %, and 20.16 %, respectively.

Cell cytotoxicity of the extracts on MCF-7 cell line

According to Figure 3 (A-C), exposure of MCF-7 cells line with WEFA resulted in reduced viability after 24- 72 hours of incubation time except for the 50 µg/mL of the WEFA after 24 hours of incubation (P= 0.26). DEFA also indicated a significant reduction in MCF-7 cell viability after 24, 48, and 72 hours of incubation for all doses. Similarly, HEFA demonstrated a significant reduction in cell viability at 24, 48, and 72 hours of incubation, except at the 50 µg/mL dose after 24 hours of incubation (P = 0.08).

According to Table 3, the IC50 values of WEFA, DEFA, and HEFA after 24 hours of incubation on the MCF-7 cell line were 186.20± 30.81, 152.40± 22.44, and 102± 16.91 µg/mL, respectively. In the comparison of different extracts, HEFA had a significantly greater cytotoxic effect than DEFA and WEFA at 100 µg/mL (P = 0.04) and 200 µg/mL (P = 0.002), respectively.

After 48 hours of incubation, the IC50 values of WEFA, DEFA, and HEFA were 131.30± 16.58, 100.30± 13.65 and 86.02± 15.36 µg/mL respectively. In comparing the different extracts, HEFA demonstrated a significantly greater cytotoxic effect than DEFA and WEFA at doses of 100 µg/mL (P = 0.04) and 200 µg/mL (P = 0.002). After 72 hours of incubation, the IC50 values of WEFA, DEFA, and HEFA were 126.30 ± 9.82 µg/mL, 87.70 ± 10.71 µg/mL, and 69.97 ± 9.45 µg/mL, respectively. Notably, HEFA exhibited significantly greater cytotoxic effects than DEFA and WEFA at doses of 100 µg/mL (P = 0.03) and 200 µg/mL (P = 0.001), respectively.

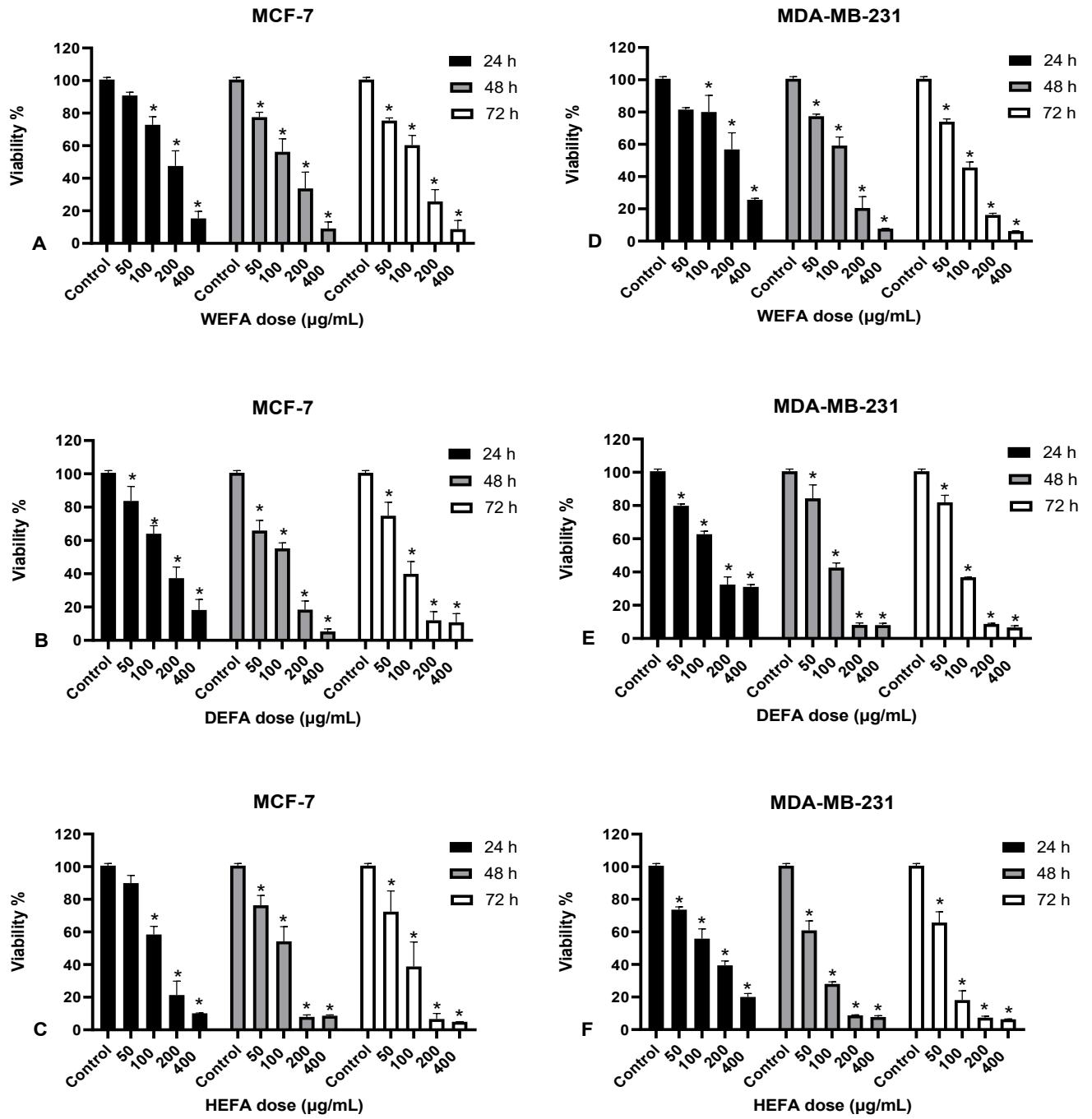

FIGURE 3. The viability of the MCF-7 (A-C) and MDA-MB 231 (D-F) cells in exposure to hydroethanolic (HEFA), dimethyl sulfoxide (DEFA), and water extract of Ferula assafoetida gum (WEFA). **P* <0.05.

Cell cytotoxicity of the extracts on the MDA-MB-231 cell line

The exposure of the MDA-MB-231 cells with WEFA, DEFA, and HEFA resulted in a significant reduction of cell viability after 24, 48, and 72 hours of incubation for all doses (Figure 3 (D-F)) except the 50 µg/mL of the WEFA after 24 hours of incubation (*P*= 0.05).

The IC50 value of WEFA, DEFA, and HEFA on the MDA-MB-231 cell line after 24 hours of incubation was 236.60± 37.44, 137.20± 64.10, and 185± 60.91 µg/mL respectively (Table 3). In comparison to the other extracts, HEFA exhibited the greatest cytotoxic effect at concentrations of 50 µg/mL (*P* = 0.007), 100 µg/mL (*P* = 0.04), and 400 µg/mL (*P* = 0.013). In the 200 µg/mL dose, DEFA exhibited the greatest cytotoxic effect (*P* = 0.011). After 48 hours of incubation, the IC50 val-

ues of WEFA, DEFA, and HEFA were 114.40 ± 5.48, 85.80 ± 4.33, and 61.26 ± 6.17 µg/mL, respectively. In comparison, HEFA demonstrated a significantly greater cytotoxic effect than DEFA and WEFA at concentrations of 50 µg/mL (P = 0.023) and 100 µg/mL (P = 0.001). After 72 hours of incubation, the IC50 values of WEFA, DEFA, and HEFA were 86.77 ± 40.59, 83.48 ± 2.61, and 60.22 ± 2.37 µg/mL, respectively. In comparison, HEFA exhibited a significantly greater cytotoxic effect than DEFA and WEFA at concentrations of 100 µg/mL (P = 0.005) and 200 µg/mL (P = 0.003).

Discussion

Ferula assafoetida contains a wide range of phenolic compounds and flavonoids, each with unique characteristics and health benefits. Solvent selection affects the types and quantities of phenolic compounds and flavonoids extracted. Phenolic compounds and flavonoids exhibit potent antioxidant and anti-cancer properties (Panahi et al., 2020). Different solvents can target specific classes or subclasses of phenolic compounds and flavonoids, allowing researchers to tailor the extraction process to their desired compounds of interest.

The present study indicates that hydroethanol was the best solvent for the extraction of phenolic compounds and flavonoids of *Ferula assafoetida*. The phenolic compounds extracted by hydroethanol were 41.90 and 69.55 percent higher than that of DMSO and aqueous solvents respectively. The flavonoids extracted by hydroethanol also respectively were 37.98 and 72.07 percent higher than that of DMSO and aqueous solvents. In fact, hydroethanol can offer enhanced solubility for a wide range of polar and moderately polar compounds. It allows for the extraction of both hydrophilic and lipophilic compounds due to the presence of water and ethanol, making it versatile for targeting various phenolic compounds and flavonoids (Koffi et al., 2010).

The higher efficiency of the hydroethanol in the extraction of phenolic and flavonoid compounds of *Ferula assafoetida* can also be attributed to its polarity. The hydroethanol solvent has a higher polarity compared to DMSO and a lower polarity compared to aqueous. In fact, hydroethanol provides a balance between the polar and nonpolar properties required for the efficient extraction of phenolic compounds and flavonoids from *Ferula assafoetida*. These compounds have hydroxyl groups (-OH) in their structures and their polar properties are dominant. Therefore, phenolic compounds and flavonoids can interact with the polar solvent molecules of hydroethanol more efficiently (Dent et al., 2013).

The previous studies indicated the efficiency of hydroethanol or ethanol for the extraction of phenolic and flavonoid compounds of *Ferula assafoetida* (Ebrahim Latifi et al., 2021; Yazdanipour et al., 2021). In the present study, the extracted phenolic and flavonoid compounds and flavonoids by the hydroethanol solvent were substantially higher than those of previous studies. Niazmand et al. reported the extracted phenolic and flavonoid compounds of *Ferula assafoetida* respectively as 9.67± 0.45 µg GAE/mg and 0.11± 0.02 µg QE/mg after 3 hours of maceration in ethanol 80% (Razieh Niazmand et al., 2021). Yazdanipour et al. also reported the extraction of 29.5 µg GAE/mg and 6.1 µg QE/mg phenolic and flavonoid compounds after 48 hours of maceration with ethanol 50% (Yazdanipour et al., 2021). It appears that the maceration time should be extended to 7 days for more efficient extraction of phenolic and flavonoid compounds of *Ferula assafoetida* (Dent et al., 2013).

In the present study, DMSO was more effective than water in extracting phenolic compounds and flavonoids of *Ferula assafoetida*. The phenolic compounds extracted by DMSO were 47.59 percent higher than water. The flavonoids extracted by DMSO were also 54.96 percent higher than water. The higher efficiency of DMSO is probably attributed to its ability to extract both polar and non-polar compounds. According to previous studies, some of the phenolic compounds and flavonoids of *Ferula assafoetida* (ferulic acid, umbelliferone, coumarin, vanillic acid, quercetin, rutin, isorhamnetin, and kaempferol) have also lipophilic properties (Ghaffari Sirizi et al., 2023). These compounds may be poorly soluble or insoluble in water. DMSO's lipophilic nature allows for the effective extraction of lipophilic phenolic compounds that may not be efficiently extracted using water as a solvent.

Hydroethanol as the most efficient solvent in the extraction of phenolic and flavonoid compounds of *Ferula assafoetida* has also indicated high antioxidant activity. The hydroethanolic extract of *Ferula assafoetida* has also higher cytotoxic effects on MCF-7 and MDA-MB-231 breast cancer cell lines. It appears that the higher phenolic compounds and flavonoids extracted by hydroethanol solvent probably strengthen the antioxidant

activity and oxidative stress. Oxidative stress is also one of the effective arms in the treatment of breast cancer.

It is important to compare the cytotoxic effects of herbal extracts by the existing criteria. The herbal extracts with the IC50 value of 20-100 µg/mL and 100-1000 µg/mL are considered respectively as "relatively active" and "weakly active" compounds (Baharum et al., 2014). The IC50 values of the cytotoxic effect of HEFA on MCF-7 and MDA-MB-231 cell lines after 72 hours of incubation respectively were 69.97± 9.45 and 60.22± 2.37 µg/mL. Therefore, the hydroethanolic extract of *Ferula assafoetida* is classified as a relatively active cytotoxic compound. In line with the trend of changes in extracted phenolic and flavonoid contents and antioxidant effects, the cytotoxic effects of DEFA were also lower than HEFA. The IC50 values of the cytotoxic effect of DEFA on MCF-7 and MDA-MB-231 cell lines after 72 hours of incubation were 87.70± 10.71 and 83.48± 2.61 µg/mL, respectively. Therefore, the DEFA is also classified as a relatively active cytotoxic compound but weaker than HEFA. The IC50 value of cytotoxic effects of WEFA on MCF-7 and MDA-MB-231 cell lines after 72 hours of incubation respectively were 126.30± 9.82 and 86.77± 4.59 µg/mL. Therefore, the cytotoxic effects of WEFA were lower than those of DEFA and HEFA and were considered as a weakly active compound on MCF-7 and relatively active on the MDA-MB-231 cell line.

Numerous studies reported the cytotoxic effects of different extracts of *Ferula assafoetida*. In a study, the effects of *Ferula assafoetida* on hypoxia-induced human umbilical vein endothelial cells (HUVECs) were observed, resulting in angiogenesis induction through the upregulation of VEGFR-1 and downregulation of VEGF genes. (Yazdanipour et al., 2021).

Ferula assafoetida leaf extract has exhibited higher amounts of flavonoid and phenolic compounds highlighting the higher potential of DPPH scavenging and ferric-reducing power. The extract also demonstrated antibacterial effects (Niazmand et al., 2021). *Ferula assafoetida* ethanolic extract at concentrations of 10, 50, 100, and 200 µg/mL caused morphological changes in the HepG2 cell line after 24 hours. However, morphological alterations in normal cells were also observed at concentrations of 100 and 200 µg/mL, which also led to a reduction in L929 cell viability (Sadooghi et al., 2013).

Various cell lines, such as PC12, MCF-7, MDA-MB-231, and 4T1 (mouse), have been inhibited upon exposure to the ethanolic extract. Moreover, its methanolic and ethanolic extracts at 20 mg for 48 hours exerted the highest anticancer effects against the osteosarcoma cell line (Panwar et al., 2015). Moreover, the petroleum and chloroform extract had IC50 of <52 µg/mL against MDBK, HT-29, A549, MCF7, and HepG2 cell lines (Mosaddegh et al., 2012). Additionally, its hydroalcoholic extract mitigated the mRNA expression of vimentin, Snail1, and Zeb1 transition markers and Bcl2, CD44, and CD54 genes (Keyghobadi et al., 2022). The IC50 of *Ferula persica* and *Ferula hezarlalezarica* against A549, HT29, HepG2, and MCF7 cells ranged from 22.3-71.8 µg/mL and 76.7 to 105.3 µg/mL, respectively (Esmaeili et al., 2012).

In addition, the cancer chemopreventive properties of related terpenoid coumarins have been demonstrated in Raji cells at concentrations of less than 10 nM (Iranshahi et al., 2008). *Ferula assafoetida* has been shown to decrease mammary gland growth and the size of palpable mammary tumors (Mallikarjuna et al., 2003). An in vivo assessment revealed that *Ferula assafoetida* oleo gum resin at 100 mg/kg decreased tumor size and volume, causing necrosis in 4T1 cells of BALB/c mice (Bagheri et al., 2017). In another study, EOs of *Ferula assafoetida* changed the TNF-α, caspase-3, TGF-β, and NF-kB signaling pathways (Verma et al., 2019). Additionally, its EOs inhibited the growth of MCF7 cells without causing any change in hematological and biochemical alterations in Wister rats (Bagheri et al., 2020).

Therefore, it appears that the hydroethanolic extract of *Ferula assafoetida* has a high potential as an anti-cancer drug. It is suggested that the cytotoxic effects of the hydroethanolic extract on normal cell lines be evaluated in future studies. Although *Ferula assafoetida* has been used in traditional medicine and among the general public, comprehensive studies on its toxicity have not been conducted. Therefore, it is suggested to investigate the effects of *Ferula assafoetida* on liver enzymes and kidney indicators in humans.

Conclusion

Hydroethanol (70% v/v) was the best solvent for the extraction of phenolic compounds and flavonoids from *Ferula assafoetida* due to its versatile properties in extracting both hydrophilic and lipophilic compounds. The antioxidant activity and cytotoxic effects of the hydroethanolic extract of *Ferula assafoetida* were also

higher than those of DMSO and aqueous extracts. The cytotoxic effects of HEFA on MCF-7 and MDA-MB-231 cell lines exhibited a concentration- and time-dependent pattern. The IC50 values of HEFA's cytotoxic effects on MCF-7 and MDA-MB-231 after 72 hours of incubation were 69.97 ± 9.45 µg/mL and 60.22 ± 2.37 µg/mL, respectively, classifying it as a relatively active anticancer compound.

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Conflict of interest

The researchers declare that there is no conflict of interest.

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