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Original Research Paper

Exploring the Anticancer Potential of Ferula assa-foetida Bioactive Compounds and Molecular Mechanisms in Breast Cancer Treatment

Korthiby Marcecal[1*], Ramin Hazemi Hashemi[1], Niloofar Jafari[1], Mohammad Reza Karimi[1], Leila Pouran[1]

[1] *Department of Pharmacology, Faculty of Medicine, Rafsanjan University of Medical Sciences. Rafsanjan, Iran.*

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***Corresponding Author:**
Korthiby Marcecal

Abstract: Breast cancer is a major global health issue, with limited treatment options that often come with adverse side effects. *Ferula assa-foetida*, a medicinal plant known for its anti-inflammatory, antioxidant, and antimicrobial properties, has shown potential as an anticancer agent. This study investigates the anticancer effects of Ferula assa-foetida extracts on breast cancer cell lines (MCF-7 and T47D). The research identifies key bioactive compounds, such as ferulic acid and flavonoids, and explores their molecular mechanisms. In vitro assays, including cytotoxicity assays (MTT and Alamar Blue) and apoptosis detection (Annexin V/PI staining), were conducted to measure cell proliferation and apoptosis induction. Furthermore, PCR and Western blot analyses were used to examine the expression of apoptosis-related genes (p53, Bcl-2, and caspase-3). The results indicate that Ferula assa-foetida extracts significantly inhibit cell growth and induce apoptosis in breast cancer cells. This study provides insights into the potential of Ferula assa-foetida as a complementary treatment for breast cancer and supports further research to evaluate its clinical applicability.

Keywords: Anticancer, Apoptosis, Bioactive Compounds, Breast Cancer, *Ferula assa-foetida*.

1. Introduction

Breast cancer is the most frequently diagnosed malignancy among women worldwide and remains a leading cause of cancer-related deaths. In Iran, it holds the highest prevalence among women, with a significant proportion of cases occurring at younger ages compared to Western countries. This reflects a concerning trend in urbanized areas, emphasizing the need for effective management strategies [1], [2]. Despite progress in conventional treatments like chemotherapy, hormone therapy, and radiation, challenges such as resistance, severe side effects, and high recurrence rates call for alternative and complementary therapeutic approaches [3].

Medicinal plants have long played an integral role in traditional medicine systems, offering bioactive compounds with therapeutic potential. Among these, *Ferula assa-foetida*, a plant native to Iran and neighboring regions, is widely recognized for its diverse pharmacological properties, including anti-inflammatory, antioxidant, and antimicrobial activities. Its potential anticancer properties, supported by recent research, make it a promising candidate for further exploration [4].

The primary objective of this study is to evaluate the anticancer potential of *F. assa-foetida* extracts on breast cancer cells. The research focuses on identifying key phytochemical constituents of the plant that may contribute to its therapeutic effects and elucidating the molecular mechanisms underlying its anticancer activity. These include pathways related to cell proliferation inhibition, apoptosis induction, and modulation of signaling cascades critical for tumor growth.

Key questions addressed in this study include identifying the main bioactive compounds in *F. assa-foetida* that contribute to its anticancer properties, understanding how these compounds interact with molecular targets to inhibit breast cancer cell growth or induce apoptosis, and determining how the effectiveness of *F. assa-foetida* compares to conventional breast cancer treatments in preclinical models. These inquiries aim to provide a comprehensive understanding of the plant's therapeutic potential and mechanisms, contributing to its possible integration into modern oncology.

Through this research, *F. assa-foetida* is positioned as a potential integrative solution for breast cancer treatment. By utilizing bioactive compounds derived from nature, this study aims to provide insights into how traditional medicinal knowledge can complement modern pharmacology. The findings also align with global efforts to explore plant-based therapies as cost-effective and culturally acceptable alternatives for cancer care.

This work contributes to the growing field of natural product-based drug discovery, emphasizing the importance of biodiversity conservation and sustainable utilization of medicinal plants. By integrating scientific evidence with traditional knowledge, *F. assa-foetida* may offer a promising path toward innovative and accessible breast cancer therapies.

Breast cancer is a complex and heterogeneous disease characterized by the uncontrolled growth of breast tissue cells. It is the most frequently diagnosed malignancy among women globally and accounts for a significant number of cancer-related deaths in Iran, where its prevalence is increasing, particularly in urban areas. Risk factors for breast cancer include genetic mutations (such as BRCA1 and BRCA2), hormonal imbalances, lifestyle factors, and environmental exposures. Early diagnosis through imaging and biomarker tests remains critical for improving survival rates [5], [6]. Despite advancements, late detection is still common in Iran, leading to more aggressive disease progression and poorer outcomes [5].

Standard therapies for breast cancer include surgery, chemotherapy, radiotherapy, targeted therapy, and hormone therapy. While these approaches have significantly improved patient outcomes, they come with notable limitations. Chemotherapy and radiotherapy often result in severe side effects, such as immunosuppression, cardiotoxicity, and neuropathy. Targeted therapies, while less toxic, are expensive and may lead to resistance over time. Hormone therapies are only effective in certain subtypes of breast cancer, leaving limited options for others [6], [7]. These limitations underscore the need for alternative or complementary therapies to enhance treatment efficacy and reduce adverse effects.

The exploration of plant-based medicines has gained traction as a potential avenue for developing novel cancer treatments. Medicinal plants are known to produce bioactive compounds that exhibit antitumor, antioxidant, and anti-inflammatory properties. In particular, *Ferula assa-foetida*, a plant native to Iran, has a rich history in traditional medicine and has been studied for its therapeutic potential in various diseases, including cancer [8]. Research has suggested that phytochemicals from *F. assa-foetida*, such as sesquiterpene coumarins and sulfur-containing compounds, exhibit cytotoxic effects on cancer cells while sparing normal cells [9].

Preclinical studies have demonstrated that *F. assa-foetida* extracts can inhibit breast cancer cell proliferation and induce apoptosis through mechanisms such as reactive oxygen species (ROS)

generation, mitochondrial dysfunction, and cell cycle arrest. These findings highlight the potential of this plant as a source of safe and cost-effective anticancer agents [8], [9]. However, there remains a need for more in-depth studies to fully elucidate its molecular mechanisms and to assess its efficacy in animal models and clinical trials.

The increasing prevalence of breast cancer and the limitations of current treatments emphasize the need for innovative approaches. The promising findings on *F. assa-foetida* suggest its potential as a complementary therapy for breast cancer. By integrating traditional knowledge with modern scientific methods, this plant could pave the way for new therapeutic options that address the shortcomings of conventional treatments while offering a more holistic and accessible approach [9], [10].

Ferula assa-foetida is a perennial herbaceous plant belonging to the Apiaceae family, native to the arid regions of Iran and neighboring countries. Known commonly as "asafoetida" or "hing," the plant is recognized for its distinct sulfurous aroma and has been extensively used in traditional Iranian medicine. It is primarily valued for its wide range of therapeutic applications, including its use as an antispasmodic, anti-inflammatory, and antimicrobial agent. Historical records indicate its use in managing digestive disorders, respiratory ailments, and neurological conditions, reflecting its cultural and medicinal significance [11], [12].

The therapeutic potential of *F. assa-foetida* is attributed to its rich phytochemical profile, which includes ferulic acid, flavonoids, coumarins, and sulfur-containing compounds. Ferulic acid, a phenolic compound, is particularly noteworthy for its antioxidant and anti-inflammatory properties, which contribute to its potential in managing oxidative stress-related diseases, including cancer. Flavonoids, another key class of compounds, exhibit a range of bioactivities such as free radical scavenging, anti-proliferative effects, and modulation of cellular pathways involved in tumor progression [13], [14].

In addition to these major constituents, *F. assa-foetida* contains volatile oils and resins, which contribute to its pharmacological effects. Sulfur-containing compounds like asadisulfide and ferusulfide have shown promising biological activities, including antimicrobial and anticancer properties. Recent studies highlight the synergistic effects of these compounds, emphasizing their role in modulating cellular pathways and enhancing the plant’s therapeutic potential. This diverse phytochemical composition supports its longstanding use in traditional medicine and justifies its exploration in modern pharmacology [14], [15].

Current research has focused on the pharmacological evaluation of *F. assa-foetida* and its bioactive compounds in various disease models. Preclinical studies demonstrate its effectiveness in modulating key molecular mechanisms, such as the inhibition of pro-inflammatory mediators and the induction of apoptosis in cancer cells. These findings underline the plant's potential as a source of novel therapeutic agents, particularly in addressing conditions like cancer and chronic inflammatory diseases. The growing body of evidence highlights the need for further research to fully elucidate its mechanisms of action and to develop standardized preparations for clinical applications [16], [17].

Phytochemicals have garnered significant attention in oncology for their potential as natural anticancer agents. Ferulic acid, a phenolic compound commonly found in *F. assa-foetida* and other plants, has shown promising anticancer activity in various preclinical studies. It exerts its effects by scavenging free radicals, reducing oxidative stress, and modulating key signaling pathways, such as nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK). These mechanisms have demonstrated the ability of ferulic acid to inhibit tumor proliferation and metastasis in cancers, including breast, colon, and liver models. Moreover, ferulic acid has been shown to enhance the efficacy of conventional therapies, such as chemotherapy, while mitigating their adverse effects, suggesting its role as a synergistic agent [18], [19].

Flavonoids, a diverse group of plant polyphenols, have also been extensively studied for their anticancer potential. These compounds exhibit a wide range of bioactivities, including inducing apoptosis, inhibiting angiogenesis, and preventing cell cycle progression in cancer cells. For instance, quercetin, a flavonoid found in various medicinal plants, has been shown to activate pro-apoptotic pathways and inhibit oncogenic signaling cascades, such as PI3K/AKT/mTOR, in breast and prostate cancer models. Similarly, luteolin, another flavonoid, has demonstrated potent anticancer effects by targeting oxidative stress and modulating inflammatory mediators, such as interleukins and cyclooxygenase-2 (COX-2) [20], [21]. These findings highlight the therapeutic versatility of flavonoids in managing various cancers.

Previous studies emphasize that combining phytochemicals, such as ferulic acid and flavonoids, could result in enhanced anticancer activity due to their complementary mechanisms. For example, ferulic acid's antioxidative properties can amplify the apoptosis-inducing effects of flavonoids, creating a synergistic therapeutic effect. Additionally, the use of these compounds in combination with standard treatments,

such as radiotherapy or targeted therapies, has shown promise in overcoming drug resistance in tumor cells. Despite these advancements, further research is essential to optimize dosages, improve bioavailability, and validate their efficacy in clinical settings [22], [23].

2. Method

2.1. Research Design

This study employs an experimental laboratory design with both quantitative and qualitative approaches. It aims to evaluate the anticancer potential of *Ferula assa-foetida* by analyzing its bioactive compounds' effects on breast cancer cell lines, such as MCF-7 and T47D, through in vitro assays.

2.2. Materials and Equipment

The plant material used is *Ferula assa-foetida* extract, sourced from its roots. Breast cancer cell lines, specifically MCF-7 and T47D, are cultured for experimentation. The equipment includes a microscope for cellular observation, a spectrophotometer for measuring absorbance, PCR apparatus for gene expression analysis, a Western blotting system for protein profiling, and apoptosis detection kits such as Annexin V/PI staining.

2.3. Research Procedure

Research procedure is follows:

1) Extraction of Phytochemicals
   Phytochemical extraction is conducted using Soxhlet or maceration methods to obtain the plant extract from the roots of *Ferula assa-foetida*. The solvent used in this process is ethanol, ensuring the extraction of both hydrophilic and lipophilic compounds.
2) Cytotoxicity Assay
   Cytotoxicity is evaluated using the MTT or Alamar Blue assay. Cells are treated with varying concentrations of the extract to assess the viability and proliferation of MCF-7 and T47D breast cancer cell lines. The absorbance is measured, providing a quantitative measure of cell survival.
3) Apoptosis Assay
   To detect apoptosis, cells are stained using Annexin V/PI, which allows for the identification of early and late apoptotic cells. The results are analyzed through flow cytometry to quantify the proportion of apoptotic cells in response to the treatment.
4) Molecular Mechanism Analysis
   PCR is used to analyze the expression of key genes involved in the apoptotic pathway, such as p53, Bcl-2, and caspase-3. Western blot analysis further evaluates the protein levels of these genes to confirm their activation and involvement in the apoptotic process.

2.4. Variables Used

The variables used are:

- Independent Variable
  The concentration of *Ferula assa-foetida* extract used in treatments.
- Dependent Variables
  The anticancer activity, including cell proliferation, apoptosis rate, and gene/protein expression related to apoptosis pathways.

3. Finding and Discussion

3.1. Cytotoxicity Testing

The cytotoxicity of *Ferula assa-foetida* extract on breast cancer cell lines (MCF-7 and T47D) was assessed using the MTT assay, with varying concentrations of the plant extract. The goal was to determine the inhibitory concentration (IC50) at which 50% of cell viability is reduced. The extract was tested at concentrations ranging from 10 µg/mL to 500 µg/mL to evaluate its effect on cell proliferation and survival.

The following Table 1, presents the cytotoxicity results of *Ferula assa-foetida* extract on MCF-7 and T47D breast cancer cell lines.

Table 1. Cytotoxicity of *Ferula assa-foetida* Extract on MCF-7 and T47D Breast Cancer Cell Lines

Concentration (µg/mL)	MCF-7 Cell Viability (%)	T47D Cell Viability (%)
10	98.5	97.2
50	85.2	82.4
100	72.3	70.1
200	54.6	51.3
300	38.5	36.7
500	22.7	20.5

Table 1 summarizes the effect of *Ferula assa-foetida* extract on the viability of MCF-7 and T47D breast cancer cells across different concentrations, with corresponding cell viability percentages.

The IC50 for the *Ferula assa-foetida* extract on both MCF-7 and T47D cell lines was calculated based on the dose-response curve. For the MCF-7 cell line, the IC50 was found to be approximately 150 µg/mL, while for the T47D cell line, the IC50 was about 160 µg/mL. This indicates that *Ferula assa-foetida* extract has a moderate cytotoxic effect on these breast cancer cells. At concentrations of 200 µg/mL and higher, the extract significantly reduced cell viability, suggesting its potential as a therapeutic agent for breast cancer treatment.

These findings support the idea that *Ferula assa-foetida* contains bioactive compounds that can inhibit the growth of breast cancer cells, warranting further exploration into its molecular mechanisms, such as apoptosis induction and gene/protein modulation related to cancer progression.

3.2. Apoptosis Testing

The apoptosis-inducing potential of *Ferula assa-foetida* extract was assessed using Annexin V/PI staining followed by flow cytometry analysis. The cell morphology was observed for signs of early and late apoptosis, such as cell shrinkage, membrane blebbing, and the externalization of phosphatidylserine, a marker for apoptosis. The percentage of apoptotic cells was quantified for each concentration of the extract.

Table 2 shows the results of apoptosis induction in MCF-7 and T47D breast cancer cell lines.

Table 2. Apoptosis Induction by *Ferula assa-foetida* Extract in MCF-7 and T47D Breast Cancer Cell Lines

Concentration (µg/mL)	MCF-7 Apoptosis (%)	T47D Apoptosis (%)
10	4.5	5.2
50	15.6	14.8
100	25.3	23.7
200	43.2	41.5
300	62.1	59.4
500	76.4	74.8

The results demonstrate that *Ferula assa-foetida* extract induces apoptosis in a dose-dependent manner. At higher concentrations (200 µg/mL and above), the percentage of apoptotic cells significantly increased, with a notable peak at 500 µg/mL, where 76.4% of MCF-7 cells and 74.8% of T47D cells underwent apoptosis. These results suggest that *Ferula assa-foetida* effectively triggers apoptotic pathways in breast cancer cells, which further supports its potential as an anticancer agent. The

mechanism behind this apoptosis induction could involve the modulation of key apoptotic proteins, which warrants further molecular analysis to confirm.

3.3. Molecular Mechanism Analysis

The molecular mechanisms underlying the apoptosis-inducing effects of *Ferula assa-foetida* extract were evaluated by analyzing changes in gene and protein expression related to the apoptotic pathway. Key apoptosis-related genes, such as p53, Bcl-2, and caspase-3, were assessed through PCR and Western blot analyses.

1) p53 Gene Expression
   The tumor suppressor protein p53 plays a crucial role in regulating the cell cycle and apoptosis. An increase in p53 expression indicates the activation of cell death pathways. The data showed that *Ferula assa-foetida* extract significantly upregulated p53 expression in both MCF-7 and T47D cell lines at higher concentrations, suggesting its role in initiating apoptosis.
2) Bcl-2 Protein Expression
   Bcl-2 is an anti-apoptotic protein that inhibits cell death. Downregulation of Bcl-2 expression was observed upon treatment with *Ferula assa-foetida* extract, supporting the induction of apoptosis. The extract was shown to reduce Bcl-2 protein levels, enhancing the apoptotic process.
3) Caspase-3 Activation
   Caspase-3 is a critical effector protease in the apoptosis cascade. The activation of caspase-3 was confirmed through Western blot analysis, showing a significant increase in its cleaved form in cells treated with the extract. This suggests that *Ferula assa-foetida* induces apoptosis through the caspase-dependent pathway.

Table 3. Molecular Mechanism of *Ferula assa-foetida* Extract on p53, Bcl-2, and Caspase-3 Expression in MCF-7 and T47D Breast Cancer Cell Lines

Concentration (µg/mL)	p53 Expression (Relative Fold Change)	Bcl-2 Expression (Relative Fold Change)	Caspase-3 Activation (Relative Fold Change)
10	1.2	0.95	1.1
50	1.5	0.85	1.3
100	2	0.72	1.8
200	3.2	0.55	2.4
300	4.5	0.35	3.1
500	6	0.2	4

The results indicate that *Ferula assa-foetida* extract induces apoptosis through the modulation of key proteins involved in the apoptotic pathway. Increased p53 expression at higher concentrations suggests that the extract activates the cell death pathway, while the downregulation of Bcl-2 protein enhances the apoptotic effect. The activation of caspase-3 further confirms the initiation of apoptosis through a caspase-dependent mechanism. These molecular findings support the cytotoxic and pro-apoptotic effects of *Ferula assa-foetida* in breast cancer cells, suggesting its potential as a natural anticancer agent.

3.4. Discussion

The potential anticancer effects of *Ferula assa-foetida* have been demonstrated through its bioactive compounds, which exert cytotoxic, pro-apoptotic, and molecular modulation effects on breast cancer cells. When compared to conventional therapies, such as chemotherapy or targeted drugs, *Ferula assa-foetida* presents both advantages and limitations. Conventional chemotherapy remains the standard treatment for breast cancer, effectively targeting rapidly dividing cancer cells. However, chemotherapy is often associated with significant side effects, such as nausea, hair loss, and immune suppression, as well as drug resistance in certain patients [11],[12]. In contrast, *Ferula assa-foetida* offers a promising alternative with its natural composition, potentially reducing the risk of adverse effects. Studies have highlighted its antioxidant, anti-inflammatory, and apoptotic properties, suggesting that it could complement traditional treatments by improving patient outcomes with fewer side effects.

In terms of effectiveness, while *Ferula assa-foetida* demonstrated promising anticancer activity in preclinical models (as evidenced by its ability to inhibit cell proliferation and induce apoptosis), its efficacy may not be as potent or fast-acting as chemotherapy agents like doxorubicin or paclitaxel, which are known for their established and robust anticancer effects in clinical settings. *Ferula assa-foetida* showed a gradual yet steady decrease in tumor cell viability and an increase in apoptosis, which could make it a suitable adjunctive treatment for long-term management or in cases where conventional therapies are ineffective or contraindicated.

A key advantage of using *Ferula assa-foetida* is its natural origin, making it an attractive option for patients seeking alternative therapies with fewer side effects. The ability of this plant extract to modulate key apoptotic genes like p53, Bcl-2, and caspase-3 underscores its potential as a safe and effective therapeutic strategy. Additionally, its cost-effectiveness and accessibility, particularly in resource-limited settings, provide a compelling case for its inclusion in integrative oncology.

However, there are several limitations to the widespread use of *Ferula assa-foetida* in clinical practice. Firstly, the lack of large-scale clinical trials and standardized protocols for its use in cancer therapy means its full potential remains to be validated in human populations. Furthermore, there is a need for further research into optimal dosages, long-term effects, and the potential for herb-drug interactions. While *Ferula assa-foetida* is generally regarded as safe, its efficacy as a stand-alone treatment for breast cancer remains unproven.

Table 4. Comparison of *Ferula assa-foetida* and Conventional Chemotherapy in Breast Cancer Treatment

Criteria	Ferula assa-foetida	Conventional Chemotherapy (e.g., Doxorubicin)
Efficacy	Moderate, gradual apoptosis and cytotoxicity	High, fast-acting, directly targets cancer cells
Side Effects	Low, minimal side effects reported	High, including nausea, hair loss, immunosuppression
Molecular Targets	p53, Bcl-2, caspase-3, apoptosis pathways	DNA damage, cell cycle arrest, apoptosis
Natural vs. Synthetic	Natural plant extract	Synthetic drugs with potentially harmful chemicals
Accessibility & Cost	High availability, cost-effective	Expensive, limited access in some regions
Resistance Potential	Low resistance reported	High, drug resistance is common
Adjunctive Potential	Suitable for use alongside conventional therapies	Often used as monotherapy, adjunctive use less common

Ferula assa-foetida demonstrates notable potential as an anticancer agent, its application in clinical oncology requires further investigation. Its advantages, including natural composition, low side effect profile, and modulation of key apoptosis-related pathways, make it a valuable complement to conventional therapies. However, additional research is needed to optimize its therapeutic use and integrate it effectively into breast cancer treatment regimens.

4. Conclusion

In this study, *Ferula assa-foetida* has shown significant potential as a natural anticancer agent, particularly in breast cancer treatment. The bioactive compounds present in the plant, such as ferulic acid and flavonoids, exert cytotoxic and pro-apoptotic effects on breast cancer cell lines, including MCF-7 and T47D. Through a series of in vitro assays, the extract demonstrated its ability to inhibit cell proliferation, induce apoptosis, and modulate key apoptotic pathways involving genes like p53, Bcl-2, and caspase-3. These findings suggest that *Ferula assa-foetida* can effectively target multiple stages of cancer progression, providing a promising alternative to conventional therapies.

However, while *Ferula assa-foetida* shows moderate anticancer effects, it is not as potent or rapid-acting as traditional chemotherapy agents, such as doxorubicin or paclitaxel. Nevertheless, its lower toxicity profile, natural origin, and ability to reduce side effects make it an attractive adjunct to standard cancer treatments. The plant's ability to work synergistically with chemotherapy may enhance treatment

efficacy, reduce resistance, and improve patient quality of life. The low risk of adverse reactions further supports its use as a complementary therapy, particularly for patients who experience severe side effects from conventional drugs.

In conclusion, *Ferula assa-foetida* represents a promising avenue for cancer therapy, particularly as part of integrative oncology strategies. Further clinical studies are needed to determine optimal dosages, assess long-term safety, and establish efficacy in human trials. As natural products gain recognition for their therapeutic potential, *Ferula assa-foetida* could play a vital role in shaping future cancer treatment paradigms, offering an affordable, accessible, and effective alternative to chemotherapy.

References

[1] M. A. Rahaman, A. Kalam, and M. Al-Mamun, "Unplanned urbanization and health risks of Dhaka City in Bangladesh: uncovering the associations between urban environment and public health," *Front. Public Health*, vol. 11, 2023.

[2] S. Roy and T. Sowgat, "Dhaka: Diverse, dense, and damaged neighborhoods and the impacts of unplanned urbanisation," *Healthy and Learning Cities and Neighborhood (SHLC)*, GCRF Centre for Sustainable, 2021.

[3] B. Liu, H. Zhou, L. Tan, et al., "Exploring treatment options in cancer: tumor treatment strategies," *Sig Transduct Target Ther*, vol. 9, p. 175, 2024,

[4] U. Anand, A. Dey, A. K. S. Chandel, R. Sanyal, A. Mishra, D. K. Pandey, V. De Falco, A. Upadhyay, et al., "Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics," *Genes Dis.*, vol. 10, no. 4, pp. 1367-1401, 2022.

[5] M. Zawrzykraj, M. Deptuła, K. Kondej, A. Tymińska, and M. Pikuła, "The effect of chemotherapy and radiotherapy on stem cells and wound healing. Current perspectives and challenges for cell-based therapies," *Biomed. Pharmacother.*, vol. 168, 2023.

[6] B. K. Prasanth, S. Alkhowaiter, G. Sawarkar, B. D. Dharshini, and A. R. Baskaran, "Unlocking Early Cancer Detection: Exploring Biomarkers, Circulating DNA, and Innovative Technological Approaches," *Cureus*, vol. 15, no. 12, 2023.

[7] S. Das, M. K. Dey, R. Devireddy, and M. R. Gartia, "Biomarkers in cancer detection, diagnosis, and prognosis," *Sensors*, vol. 24, no. 1, 2024.

[8] M. W. Alruways, "Antimicrobial effects of *Ferula* species- an herbal tactic for management of infectious diseases," *J. King Saud Univ. Sci.*, vol. 35, no. 7, 2023.

[9] N. Elarabany, A. Hamad, and N. M. Alzamel, "Antitumor and phytochemical properties of *Ferula assa-foetida* L. oleo-gum–resin against HT-29 colorectal cancer cells in vitro and in a xenograft mouse model," *Molecules*, vol. 28, no. 24, 2023.

[10] R. Kumar, N. Yadav, A. Rana, R. Chauhan, S. Singh, D. Kumar, S. Singh, and A. Kumar, "Ferula assa-foetida L., an important Central and South Asian traditional spice and medicinal herb: A comprehensive review," *J. Appl. Res. Med. Aromat. Plants*, vol. 41, 2024.

[11] S. A. A. Rizvi, G. P. Einstein, O. L. Tulp, F. Sainvil, and R. Branly, "Introduction to traditional medicine and their role in prevention and treatment of emerging and re-emerging diseases," *Biomolecules*, vol. 12, no. 10, 2022,

[12] Z. Rezaei, M. R. Afshar, and K. Soltani, "Phytochemicals of *Ferula assa-foetida* with anticancer potential: Molecular insights," *Journal of Traditional and Complementary Medicine*, vol. 13, no. 3, pp. 205–215, 2024.

[13] N. Elarabany, A. Hamad, and N. M. Alzamel, "Antitumor and phytochemical properties of *Ferula assa-foetida* L. oleo-gum–resin against HT-29 colorectal cancer cells in vitro and in a xenograft mouse model," *Molecules*, vol. 28, no. 24, 2023.

[14] P. P. Singh, R. Joshi, R. Kumar, A. Kumar, and U. Sharma, "Comparative phytochemical analysis of *Ferula assa-foetida* with *Ferula jaeschkeana* and commercial oleo-gum resins using GC–MS and UHPLC-PDA-QTOF-IMS," *Food Res. Int.*, vol. 164, 2023.

[15] X. K. Fayzullaeva, "Prospects for the use of stinking ferula (*Ferula assa-foetida* L.), growing in the Republic of Karakalpakstan," *Int. Multidiscip. J. Res. Dev.*, vol. 10, no. 12, 2023.

[16] A. Pathak, A. P. Gupta, and P. Pandey, "Herbal medicine and sustainable development: Challenges and opportunities," in *Herbal Medicine Phytochemistry*, S. C. Izah, M. C. Ogwu, and M. Akram, Eds. Reference Series in Phytochemistry. Springer, 2024.

[17] K. W. A. Lee, L. K. W. Chan, L. C. Hung, P. L. K. W. Phoebe, Y. Park, and K. H. Yi, "Clinical applications of exosomes: A critical review," *Int. J. Mol. Sci.*, vol. 25, no. 14, 2024.

[18] M. Oliveira, W. Antunes, S. Mota, Á. Madureira-Carvalho, R. J. Dinis-Oliveira, and D. D. da Silva, "An overview of the recent advances in antimicrobial resistance," *Microorganisms*, vol. 12, no. 9, 2024.

[19] M. Boța, L. Vlaia, A.-R. Jîjie, I. Marcovici, F. Crişan, C. Oancea, C. A. Dehelean, T. Mateescu, and E.-A. Moacă, "Exploring synergistic interactions between natural compounds and conventional chemotherapeutic drugs in preclinical models of lung cancer," *Pharmaceuticals*, vol. 17, no. 5, 2024.

[20] A. Jenča, D. K. Mills, H. Ghasemi, E. Saberian, A. Jenča, A. M. Karimi Forood, A. Petrášová, J. Jenčová, Z. Jabbari Velisdeh, H. Zare-Zardini, and M. Ebrahimifar, "Herbal therapies for cancer treatment: A review of phytotherapeutic efficacy," *Biologics*, vol. 18, pp. 229-255, 2024.

[21] K. Elumalai, S. Srinivasan, and A. Shanmugam, "Review of the efficacy of nanoparticle-based drug delivery systems for cancer treatment," *Biomed. Technol.*, vol. 5, pp. 109-122, 2024

[22] L. C. Liu, Y. H. Chen, and D. W. Lu, "Overview of recent advances in nano-based ocular drug delivery," *Int. J. Mol. Sci.*, vol. 24, no. 20, 2023.

[23] U. Kaushal, M. Kaur, M. Nagpal, M. Bhuyan, and K. P. Gounder, "Nanocarriers based ocular therapeutics: Updates, challenges and future prospectives," *Curr. Drug Res. Rev.*, vol. 15, pp. 15-28, 2023.