Research Article

Phytochemical Profiling and Anticancer Effect of *Fagonia arabica* on MCF-7 Breast Cancer Cells

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Abstract

This research analyzes the phytochemicals in *Fagonia arabica* leaves, revealing a strong presence of flavonoids, terpenoids, saponins, anthraquinones, cardiac glycosides, and tannins. The methanolic fraction, rich in gallic acid and rutin equivalencies, exhibited exceptional antioxidant activity, validated through 2,2-diphenyl-1-picrylhydrazyl (DPPH) and phosphomolybdate assays. Employing FTIR and GCMS spectra, we objectively assessed the diverse chemical constituents, emphasizing the credibility of this method in identifying mix-substance systems in traditional and herbal medicine. To further understand the therapeutic potential, MCF-7 cells were treated and cytotoxicity assays (MTT and acid phosphatase assay) were conducted to get IC50 values that further confirmed the anticancer properties of *Fagonia arabica*. Real-time PCR analysis targeted Caspases and Wnt/ β-catenin genes, revealing statistically significant (***$p<0.001$) results with upregulated caspases and downregulated Wnt signaling genes. The findings highlight *Fagonia arabica* extract as a highly effective therapeutic strategy against breast cancer cells, strategically modulating Wnt/ β-catenin genes without causing significant damage to normal cells. This study shows potential in identifying novel bioactive chemicals in these medicinal plants, which will help us better grasp their therapeutic potential.

Keywords: *Fagonia arabica*, Wnt signaling, Phytochemical, Breast cancer cell lines.

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Introduction

Embryonic development relies on signaling pathways. Mutations in these pathways in adults can cause abnormal cell growth. The Wnt pathway, crucial for tissue development and cell regulation, involves Wnt ligands binding to Frizzled receptors, triggering biological responses [1, 2, 3].

Breast cancer stands as a significant cause of morbidity and mortality globally among women. Advanced methods are being developed to detect primary tumors, metastases, and recurrent disease for effective management. Research indicates that dysregulation of Wnt/ β-catenin signaling significantly fuels tumorigenesis, compromising patient survival and treatment efficacy [4]. While chemotherapy can impede tumor growth, prolonged use of single agents often yields poor results. Combination therapies are now standard, yet systemic drug delivery causes widespread damage to healthy cells, leading to adverse effects [5]. Recent studies uncover molecular pathways associated with breast cancer proliferation, metabolism, and resistance to targeted therapies [1]. Understanding these mechanisms is vital to combat innate and acquired drug resistance and enhance treatment outcomes.

Natural products and medicinal plants have long been integral to healthcare. About half of current medications are derived from natural sources, highlighting their importance. Understanding how these natural chemicals are absorbed and distributed in the body is critical [6]. Resveratrol, Curcumin, and Quercetin are a few plant-derived substances that may block the Wnt signaling pathway, which is essential for cancer development [7,8]. While salinomycin, albeit synthetic, targets cancer stem cells by blocking the Wnt pathway and shows promise for cancer treatment. Similarly, Curcumin, found in turmeric, has demonstrated anticancer capabilities in preclinical trials [9, 10]. They are still being tested for safety and efficacy in humans, emphasizing the need for thorough evaluation in cancer treatment. Given the side effects and medicine resistance associated with chemotherapy, the high rate of breast cancer in Pakistan is very concerning. The current study compares natural and herbal treatments with conventional pharmaceuticals to see which is more effective against breast cancer cell lines. One such comparison examines the possible anticancer effects of *Fagonia arabica*. Widely found across the Indian subcontinent, *Fagonia* species are tropical herbs of the Zygophyllaceae family. A major component in Ayurvedic medicine, *Fagonia arabica* is mostly used for its antihypertensive [11], anti-inflammatory [12], analgesic, and thrombolytic properties [13]. Its medicinal use depends on identifying Dhamasa via morphological and microscopic analysis [14]. For its health-promoting qualities, *Fagonia arabica* is used as a component of herbal beverages and infusions. Despite not being a common food source, folk medicine occasionally uses it as a dietary supplement to promote general health [15]. Being valued for its therapeutic qualities, and some of its parts are eaten or used as herbal remedies for their health advantages. *Fagonia arabica*, which is rich in alkaloids and flavonoids [16], may help the body combat free radicals and reduce oxidative stress [17]. This research aims to assess the effectiveness of *Fagonia arabica* targeting Wnt/ β-catenin signaling genes in MCF-7 cells, in light of its potential anticancer properties. Despite the lack of clinically approved medications that specifically target Wnt/ β-catenin signaling in cancer, there is a lot of interest in this field because several approaches are being researched.

Materials and Methods

Sample preparation

The specimens of *Fagonia arabica* collected from various locations in Pakistan have the voucher number PMAS-346. Arid Agriculture University Rawalpindi plant taxonomist Prof. Dr. Rahmatullah Qureshi recognized the specimens. The plant material was cleaned, dried in the shade for three weeks, and then ground into a powder. To guarantee a uniform particle size, it was sieved through a 60-mesh topology Willy mill, and the powder was packed within an airtight container. The preparation of the *Fagonia arabica* leaf extract (FALE) involved the use of the maceration technique [18].

Phytochemical analysis

The phytochemical analysis of the *Fagonia arabica* leaf extract (FALE) was carried out using the methodology of [19], which may have medical applications.

Antioxidant assays

To evaluate the antioxidant capability against various kinds of free radicals, two antioxidant assays were conducted.

Measuring DPPH radical scavenging activity

The assay involved serial dilution of standards and plant extract, followed by incubation in the dark with a 0.004 % DPPH stock solution with 517 nm absorption measurement [20].

TAC assay

The assay involved preparing plant extract stock solutions in DMSO, creating diluted samples, and combining them with a reagent solution, determining the total antioxidant capacity using the standard curve [19].

TPC / TFC contents

The total phenolic content of FALE was determined using gallic acid equivalents, or milligrams of gallic acid per gram of dried extract. The total flavonoid contents (mg rutin/ g dry extract) were estimated using rutin equivalents. The blank was generated from methanol using the methods given by [18].

Fourier Transform Infrared Spectro-photometer (FTIR)

FTIR spectroscopic analysis of the FALE identified many functional molecules with distinct peak values. The material was crushed in a 1:10 ratio with KBr to prepare the samples, and a hydraulic press was used to shape the material into pellets. The FTIR Spectroscopy device, which has a 400–4000 cm^{-1} scanning array and a 4 cm^{-1} resolution, was then used to analyze these pellets [21].

Gas chromatography mass spectrometry analysis

The method [21] was used to analyze the volatile components of FALE. By comparing retention periods with spectrum data from the Wiley and NIST libraries, the validity of the chemicals found by GCMS analysis was verified [22].

Cell culturing

Using the human breast cancer cell line MCF-7 (from ECACC), the study was carried out with ethical approval number IIUI/ORIC/Bioethics/110-81. The cells were cultivated grown and supplemented, according to standard growth conditions [23].

Cytotoxicity assay

MCF-7 cells were fed with Curcumin (10 mM stock) as a reference drug to evaluate FALE effects. The range of FALE administration was 0–500 µg/ml. The

methodology described by [20] was followed for both the MTT and Acid Phosphatase assays.

Selection of doses

Plant cytotoxicity tests were used to choose the doses, and they were then modified to roughly match their IC50 values. Before being processed for real-time PCR expression, MCF-7 cells received two doses of treatment.

RNA extraction and expression profiling of targeted

RNA was extracted from the treated MCF-7 cells following the method described by [24]. Subsequently, using a cDNA synthesis kit, the RNA was converted into cDNA. The expression of genes, including Caspase-1, -3, -7, -9, Wnt-3a, and β-catenin, was assessed using the cDNA as a template. The housekeeping gene GAPDH was used as a control. Expression profiling was conducted by qPCR in triplicates for all treated and untreated samples, employing the SYBR green method. The 2ΔΔ-CT technique of [25], was used to assess relative expression. Lists of the primers and the ideal PCR conditions are given in supplementary Tables.

Statistical analysis

All analyses were done in triplicate using mean ± SEM, and one one-way ANOVA was applied using Graph Pad Prism where required. Given the qualitative nature of analysis for FTIR and GCMS statistical analysis was not applicable.

Results

Percentage yield of extracts

The methanolic extract of *Fagonia arabica* yielded 18g per 100g dry powder.

Phytochemical analysis

The FALE exhibited the presence of alkaloids, phenols, flavonoids, terpenoids, coumarins, glycosides, quinones, and saponins, as shown in Table 1.

Table 1: Phytochemical analysis of selected plant extract.

Sr. no.	Phytochemical Test	FA
1	Alkaloids	+
2	Flavonoids	+
3	Phenols	+
4	Tannins	+
5	Terpenoids	+
6	Saponins	+
7	Carboxylic Acid	+
8	Cardiac Glycosides	+
9	Coumarins	+
10	Oxylates	+
11	Sterols	+

FALE= *Fagonia arabica* Leaves extract (+) = Presence of Phytochemical, (-) = Absence of phytochemical.

Antioxidant assay

The following antioxidant assays were performed to screen free radical scavengers.

DPPH Free radicals assay

The DPPH assay revealed the antioxidant potential of FALE, with results presented in Table 2. The EC50 value for FALE was determined to be 0.51 ± 0.02 mg/ ml, and dose-related activity was observed (Figure 1A). A significant correlation among all doses of the plant was detected through one-way ANOVA.

Phosphomolybdenum (Total Antioxidant capacity) assay

The phosphomolybdate assay was employed to evaluate antioxidant capacity of FALE which was measured in terms of

ascorbic acid equivalency (AEE), with results illustrated in Figure 1B and summarized in Table 3. A direct relationship was observed between the total antioxidant capacity and FALE concentration, with a decrease in AEE value with each serial dilution. Significant differences among different concentrations were determined via one-way ANOVA ($P < 0.0001$, $R^2 = 0.9368$).

TPC/ TFC

The total phenolic content of FALE was found to be 78.72 ± 0.5 mg gallic acid/ g of extract, while the total flavonoid content was measured at 50.02 ± 0.1 mg rutin/ g of extract, as illustrated in Figure 1C, 1D, and Table 4.

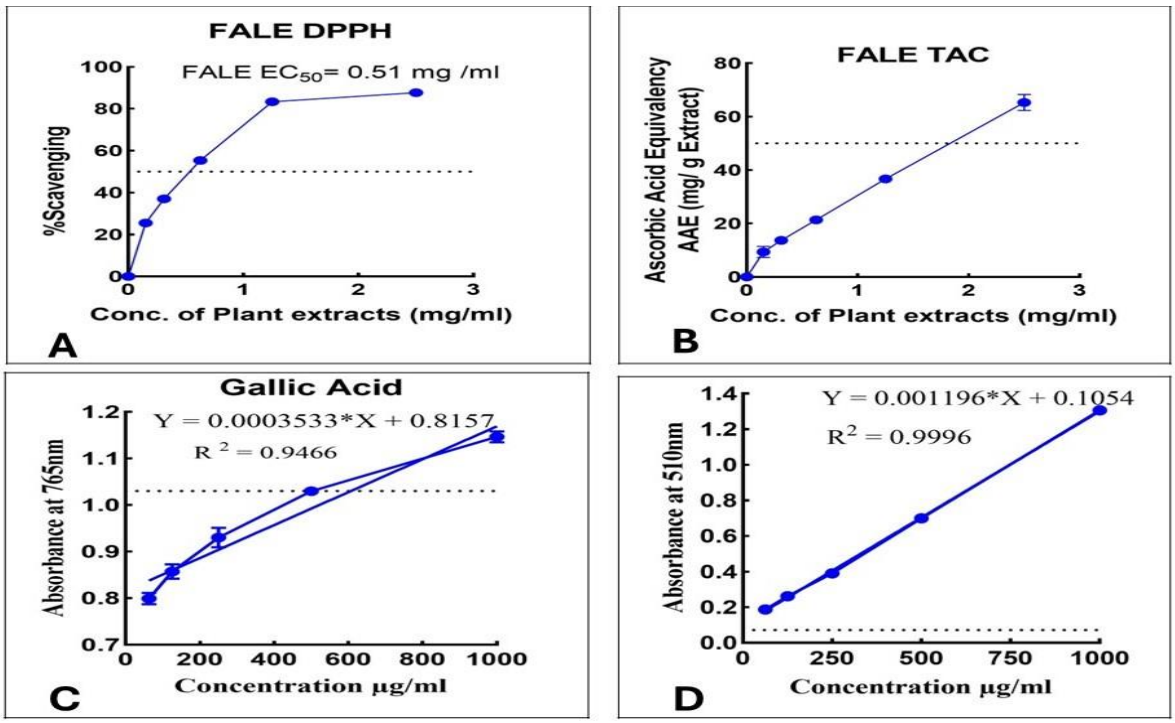


Figure 1**: A** Graphical representation of DPPH free radical scavenging activity of FALE at different concentrations. **B.** Graphical representation Total antioxidant Capacity assay for FALE at different concentrations. **C.** Graphical representations of Total Phenolic Contents of FALE using Gallic acid as a standard. **D** Total Flavonoid Contents of FALE using Rutin as a standard. Each value represents a mean ± SE (n = 3).

Table 2: Percentage scavenging (mean ± SEM) Values for *Fagonia arabica* at different concentrations.

Conc. of Samples mg/ mL	A. A	FALE
2.5	92 ± 1	88 ± 0
1.25	92 ± 0	84 ± 1
0.62	92 ± 0	56 ± 1
0.31	92 ± 0	34 ± 4
0.16	92 ± 1	29 ± 5

Values are presented as Mean ± SEM (n = 3), the percentage of DPPH free radical scavenging by A.A (Ascorbic Acid) and the methanolic extracts of FALE (*Fagonia arabica* Leaves extract).

Table 3: Phosphomolybdate assay showing ascorbic acid equivalency (AEE) mg/ g of *Fagonia arabica* extract (mean ± SEM).

Conc. of FALE (mg/ml)	Ascorbic Acid Equivalency (AEE) mg/g of *Fagonia arabica* Extract (mean ± SEM)
2.5	65 ± 2
1.25	36 ± 2
0.62	21 ± 1
0.31	13 ± 1
0.15	9 ± 1

Values are presented as Mean ± SEM (n = 3). Results of Phosphomolybdenum assay by the Methanolic plant extracts of *Fagonia arabica*.

Table 4: Quantitative equivalencies of FALE *contents* with standards.

Sample	TPC (GAE mg/ g extract)	TFC (RE mg/ g extract)
FALE	78.72 ± 0.5	50.02 ± 0.1

GAE= Gallic acid equivalence, RE= Rutin equivalency.

FTIR analysis

Our samples confirmed the presence of plants secondary metabolites through FTIR analysis, revealing functional groups present in plant extracts. Notably, peaks at specific wave numbers indicated various functional groups. For instance, the peak at 3020 cm^{-1} indicated the stretching vibration of OH, while the signal at 1709 cm^{-1} suggested HC=O stretching vibration, indicating the presence of carbonyl compounds (aldehydes). Changes in the intensity ratio of bands at 1536 cm^{-1} for CH stretching and the presence of aliphatic compounds at 1430 cm^{-1} were observed.

Additionally, peaks at 1302 cm^{-1} (C=O) indicated the presence of ketones, and 1173 cm^{-1} (C-H) signified alkanes. Furthermore, the peak at 881 cm^{-1} represented the peak of primary alcohol glucose. These FTIR results outlined the spectrum of medicinally essential plants that are beneficial for applications in the agricultural industry (Table 5, Figure 2).

Table 5: FTIR analysis showing wave numbers of functional groups identified in for FALE.

Plant extract	Peak Values cm^{-1}	Functional group	Reference
FALE	3020	H-C=O stretching	[48]
	1536	(C-H stretching) alkanes	[49]
	1302	Normal aliphatic esters	[50]
	1173	C=O stretching	[51]
	882	-OH Primary alcohol	[52]

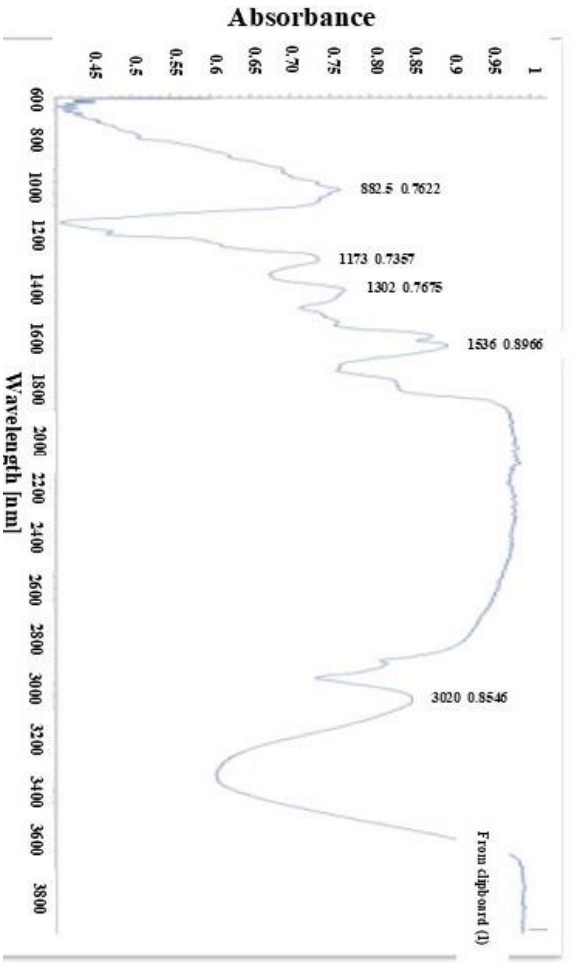


Figure 2: FTIR analysis showing functional groups of important phytochemicals present in FALE.

GC-MS analysis

GC-MS chromatograms of FALE illustrated various retention times, facilitating the identification of bioactive compounds present. By comparing mass spectra with the NIST library, 23 bioactive compounds were characterized. These compounds may contribute to the therapeutic benefits associated with the plant. Notably, 2,3-Diphenyl-5-methoxybenzoic-1,4 (58.78% abundance) and 3,7,11,15-Tetramethyl-2-hexadec (11.81% abundance) were identified as the most abundant polyphenols in FALE (Figure 3, Table 6).

Cell culturing

MCF-7 and MDA-MB-231 breast cancer cell lines were initially cultured for the study. However, due to mycobacterial contamination in MDA-MB-231, the study proceeded only with MCF-7 cell lines.

Cytotoxicity assays

To assess the cytotoxic effects of essential phytochemicals, present in FALE, MTT, and Acid Phosphatase (APT) assays were conducted. As per previous studies, a dose range of 0 to 500 µg/ml was utilized for the cytotoxicity assays.

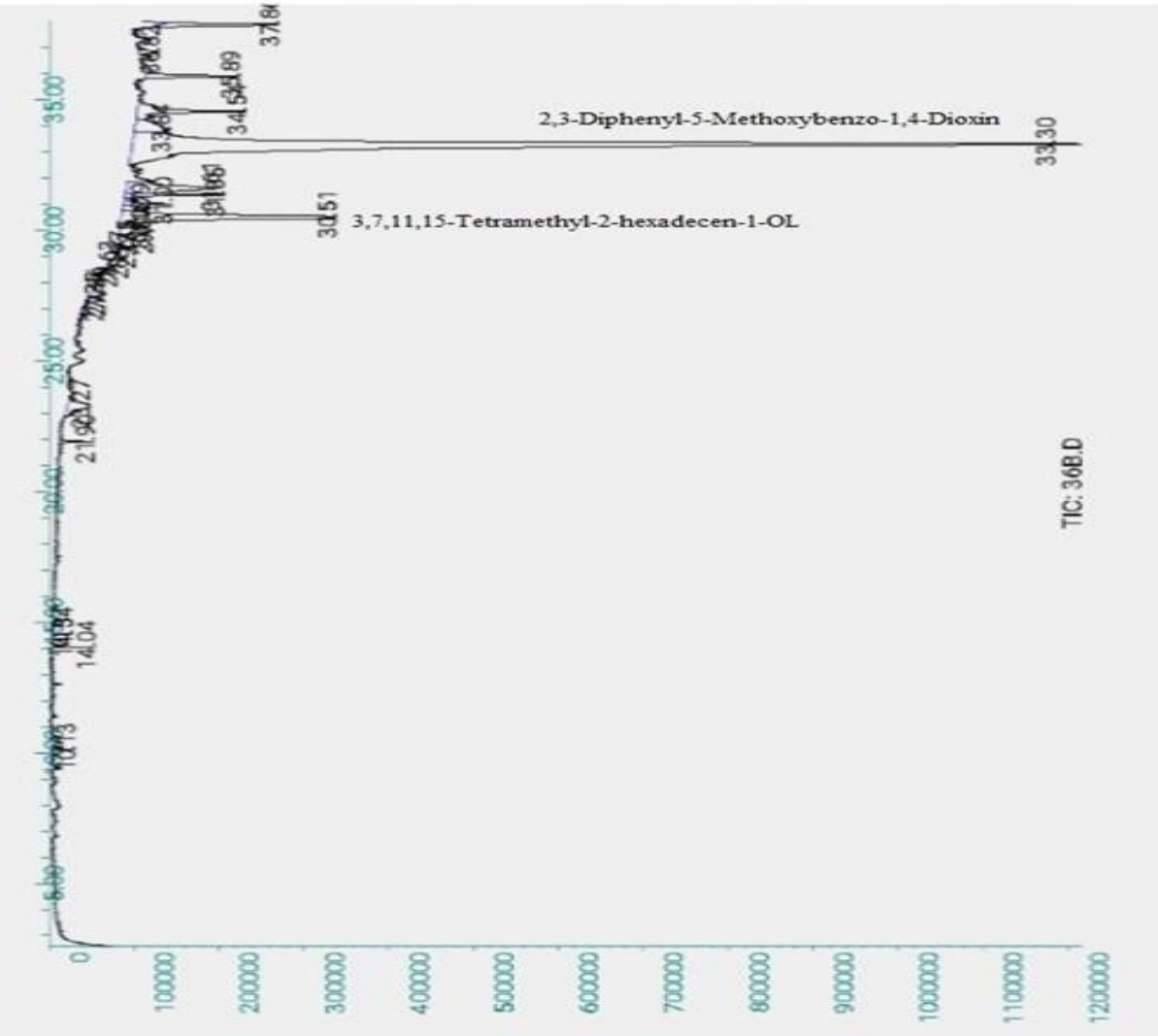
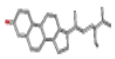
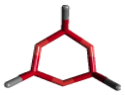
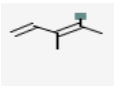
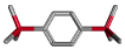
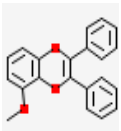
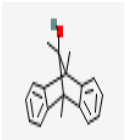
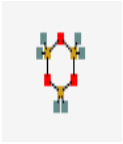
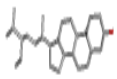
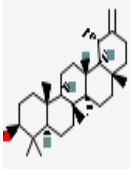


Figure 3: GCMS analysis Showing peaks for the presence of volatile components present in FALE.

Table 6: GCMS analysis of *F. arabica* showing bioactive compounds.

RT	Compound name	CAS No.	Abundance	Mol. formula	Mol. Weight	Compound Structure	Bioactivity	Ref
23.27	Stigmast-4-en-3-one	001058-61-383	2.04	C29H48O	412.7		Hypoglycemic	[52]
29.94	Hexamethyl Cyclo-trisiloxane	000541-05-943	1.36	C6H18O3Si3	222.46		antioxidant and antidiabetic activities	[53]
30.52	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	102608-53-7	11.81	C20H40O	296.5		Anti-cancerous, anti microbial, antidiuretic anti oxidant	[53]
31.35	Stigmastan-3,5-diene	1000214-16-4	2.17	C6H10	82.14		Antimicrobial	[54]
31.61	Silane, 1,4-phenylenebis	013183-70-5	5.66	C12H22Si2	222.47		Antibacterial,anti larvacidal, antiviral,anti cancerous	[55]
33.30	2,3-Diphenyl-5-Methoxybenzo-1,4-Dioxin	091201-56-8	58.78	C21H16O3	316.3		Anticancerous,	[56]
33.84	9,10-Methanoanthracen-11-Ol	084783-02-8	2.69	C18H18O	250.3		Antitumor	[57]
34.54	Cyclot-risiloxane	000541-05-9	4.44	H6O3Si3	138.3		antimicrobial	[58]
35.89	β-Sitosterol	000083-46-5	2.10	C29H50O	414.7		Anti-inflammatory antipyretic	[59]
37.87	Taraxasterol	001059-14-9	2.99	C30H50O	426.7		Anti-inflammatory, chemo-preventive	[60]

MTT and APT assay

The impact of FALE on MCF-7 cells was evaluated using MTT and acid phosphatase assays. The dosage was determined based on the initial antioxidant analysis, ranging from 0 to 500 µg/ ml. After incubating the cells with test samples for 48 hours, the percentage inhibition results were determined. Curcumin was used as a standard natural drug to compare with the samples under study via MTT and APT assays. The IC50 values for all samples are presented in Table 7 and Figure 4.

Table 7: Cytotoxicity analysis of FALE

Sample	MTT assay IC 50 (µg/ ml)	APP assay IC 50 (µg/ ml)
FALE	181 µg/ ml	109 µg/ ml
Curcumin	13.7 µg/ ml	11.5 µg/ ml

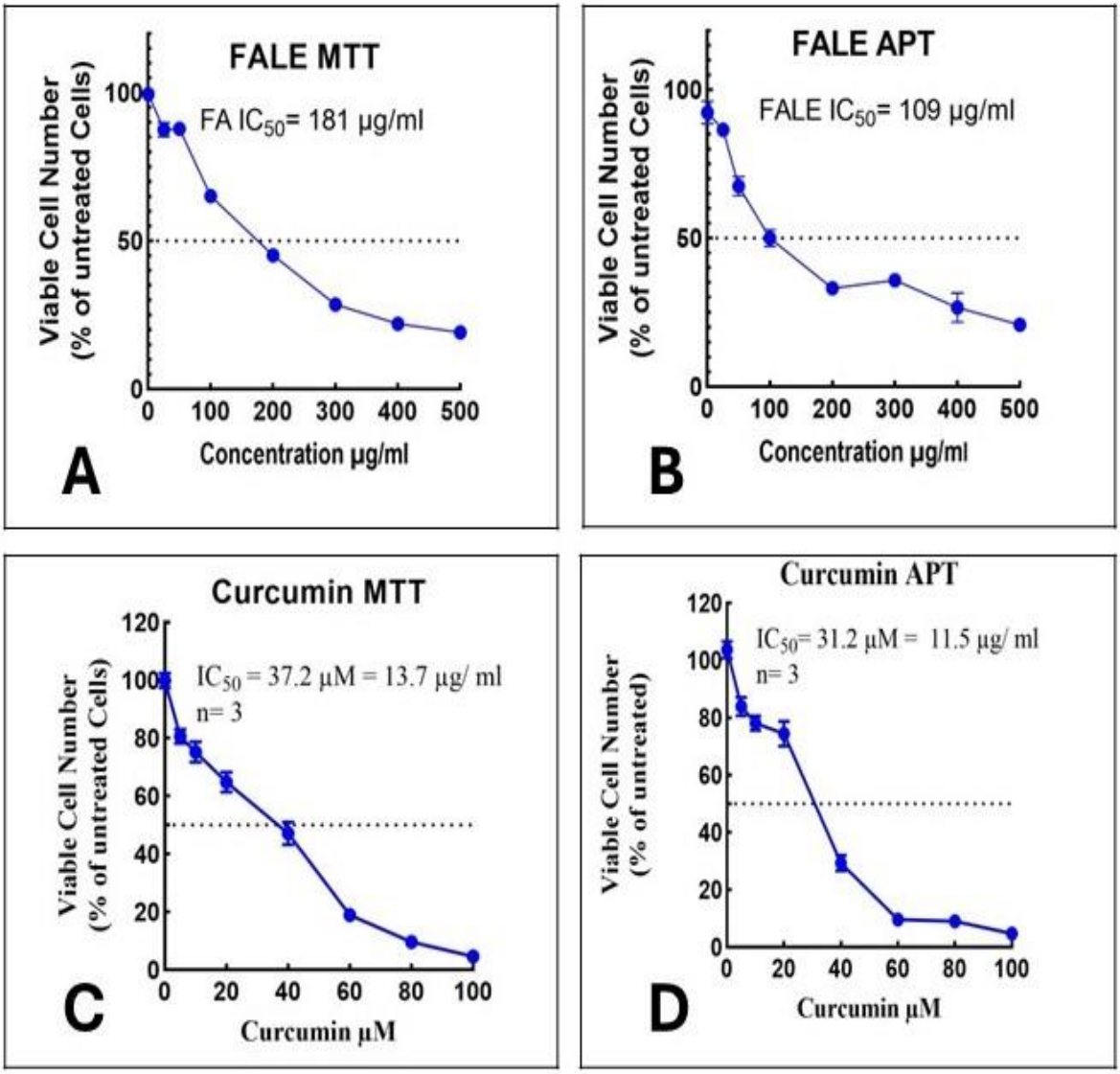


Figure 4: The percentage of MCF-7 cell death by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay and Acid phosphatase assay (APP) at different doses of FALE after 48h of incubation. **A**. MTT assay with FALE IC50= 181 µg/ml **B**. APT assay with FALE IC50= 109 µg/ml **C**. MTT assay with Curcumin IC50 = 37.2 µM = 13.7 µg/ ml **D.** APT assay with Curcumin IC50= 31.2 µM =11.5 µg/ ml. Curcumin is used as a standard drug. Each value represents a mean ± SD (n = 3).

qPCR of targeted genes by FALE

Caspase-1, -3, -7, and -9 gene expression was evaluated in MCF-7 cells treated with FALE.

In cells treated with FALE, the ΔΔCt mean of the Caspase-1 gene compared to GAPDH at 100 µg/ml was 1.86 ± 0.95, and at 300 µg/ml was 2.59 ± 0.25 with no statistical difference between two doses and with the positive control Doxorubicin at 0.75 µg/ml. In contrast, the untreated MCF-7 cells exhibited a lower ΔΔCt mean of the Caspase-1 gene, which was highly statistically significant. The ΔΔCt mean of the Caspase-3 gene in cells treated with FALE at 100 µg/ml was 2.98 ± 0.98, and at 300 µg/ml, it was 3.32 ± 0.57 with no statistical differences among both doses but revealed a significant difference with positive control (Figure 5A, Table 8). Untreated MCF-7 cells exhibited a much lower ΔΔCt mean of the Caspase-3 gene, which was highly significant statistically (Figure 5B, Table 8). The Caspase-7 gene in cells treated with FALE at 100 µg/ml was 2.32 ± 0.97, and at 300 µg/ml was 2.42 ± 0.25 and with of the Caspase-9 gene in cells treated at both doses were 2.63 ± 0.92 and 3.34 ± 0.44 respectively with statistical results similar to Caspase 3 (Figure 5C, 5D, Table 8).

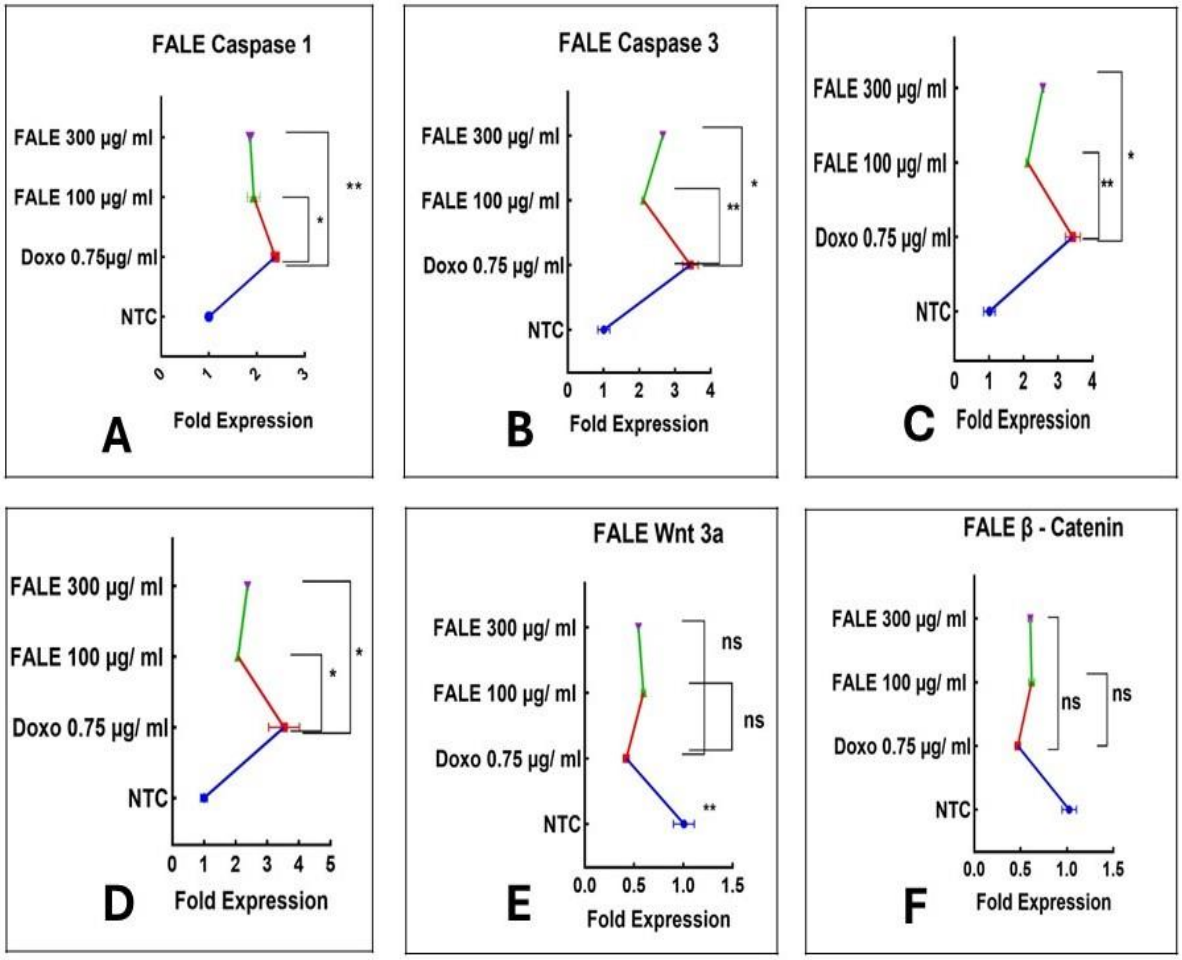


Figure 5 Comparative analysis in the expression of Caspases and Wnt genes compared to GAPDH in MCF-7 cells after 24 hr exposure with FALE. NTC: Non-treated cells, Doxo: Doxorubicin, a positive control, FALE: *Fagonia arabica* leaf extract. Comparing treated cells VS Non treated. All targeted genes showing Statistically significant difference of p **< 0.001 and p**<0.01. A Casp- 1 gene B Casp- 3 gene C Casp- 7 gene D Casp- 9 gene E Wnt- 3a gene F β- catenin gene.

Table 8: Summary of fold expression of targeted genes by FALE (Real-time PCR) (n= 6).

Treatments	2^(- ΔΔ Cq) Fold expression targeting Caspases and Wnt signaling genes					
	Casp 1	Casp 3	Casp 7	Casp 9	Wnt- 3a	β-Catenin
NTC	1.00 ± 0.04	1.01 ± 0.17	1.04 ± 0.41	1.00 ± 0.01	1.02 ± 0.28	1.00 ± 0.04
Doxorubicin 0.75 µg/ ml	2.38 ± 0.03	3.44 ± 0.22	3.26 ± 0.05	3.52 ± 0.48	0.14 ± 0.007	0.47 ± 0.02
FALE 100 µg/ ml	1.86 ± 0.95	2.98 ± 0.98	2.31 ± 0.97	2.64 ± 0.92	0.55 ± 0.93	0.41 ± 0.88
FALE 300 µg/ ml	2.59 ± 0.25	3.32 ± 0.57	2.43 ± 0.25	3.35 ± 0.44	0.35 ± 0.00	0.59 ± 0.03

The study observed a decrease in the expression of Wnt-3a and β-catenin genes following FALE compared to non-treated cells. The relative expression of the Wnt-3a gene at FALE 100 µg/ ml was 0.55 ± 0.94, and at 300 µg/ ml was 0.34 ± 0.00 and of of β-catenin gene at FALE 100 µg/ ml was 0.42 ± 0.88, and at 300 µg/ ml was 0.58 ± 0.03 with no statistical difference. Similarly, comparison with Doxorubicin as the positive control revealed non-significant differences. In contrast, untreated MCF-7 cells exhibited a higher ΔΔCt mean of the Wnt-3a gene and β-catenin gene at, which was statistically significant (Figure 5E, 5F Table 8). Overall, our results indicate a significant difference in the expression of all target genes in treated versus non-treated cells, suggesting upregulation of caspases and downregulation of Wnt-3a and β-catenin genes in MCF-7 cells upon treatment with FALE at two different doses. FALE shows potential as an effective anti-apoptotic and anticancer agent against MCF-7 cells through active gene modulation.

Discussion

Breast cancer incidence is on the rise globally due to factors such as urbanization, poor dietary habits, and the adoption of Western lifestyles, particularly in developing countries [26]. Despite advancements in chemotherapy, many cancer cells resist conventional treatments, prompting researchers to explore natural alternatives with fewer side effects [27]. Polyphenols, abundant in plants, exhibit antioxidant properties that mitigate oxidative stress by scavenging free radicals and inhibiting lipid peroxidation [28]. Our investigation utilized crude plant extract, a common practice for assessing antioxidant activity and identifying bioactive components [29]. Crude extract antioxidant combinations can have complementary, antagonistic, or additive effects [29]. Methanolic extracts were chosen for research due to their higher yield. *Fagonia arabica*, a plant with traditional, ecological, therapeutic, and economic value, was evaluated for potential pharmaceutical use. The phytochemicals of *Fagonia arabica*, including primary metabolites that neutralize free radicals and secondary metabolites that prevent ROS formation, suggest its medicinal potential [30].

Plants contain various phytochemical families with therapeutic uses, including tannins, phenols, flavonoids, alkaloids, saponins, and other compounds. Tannins are polyphenolic substances with antiviral, antifungal, and antiparasitic properties, while flavonoids and phenols have antioxidant functions and are linked to cancer and inflammation. Alkaloids have anticancer action and are being studied for breast cancer treatment. The chemical makeup of medicinal herbs is influenced by species, growth conditions, age, and harvesting period [31]. Reactive oxygen species (ROS) are a therapeutic target in

tumor progression due to their fluctuating levels. Natural antioxidants like polyphenols, flavonoids, and carotenoids, found in medicinal plants, have been studied for suppressing ROS-producing enzymes, which are sometimes overexpressed in cancer. High intake of flavonoids has been linked to treating various diseases due to their antioxidant properties and electron transfer capacity [32]. Gallic acid and rutin were used as standards to quantify the total phenolic and flavonoid contents of FALE. According to our research, FALE has 50.02 ± 0.1 mg of rutin and 78.72 ± 0.5 mg of gallic acid/ gram of extract, which closely agrees with data from earlier studies [33] signifies the antioxidant potential of FALE.

FTIR spectroscopy has become the method of choice in herbal medicine, often used more frequently than chromatographic techniques [34]. The capability of FTIR to concurrently separate several elements in comparable samples with a single experimental measurement is a powerful feature [35]. The identification of functional groups in bioactive compounds has been verified using FTIR research. It has been established that *Fagonia arabica* contains functional groups such as phenols, flavonoids, alkaloids, and terpenoids. These compounds may have pharmacological activity and potential functions in antioxidant and anticancer effects [21, 22, 36]. Numerous volatile and nonvolatile compounds can be found in plant extracts. In our investigation, 23 bioactive chemicals were identified using GC-MS analysis of FALE. Of these compounds, the two most abundant polyphenols in FALE were 2,3-Diphenyl-5-methoxybenzoic-1,4 (with a percentage abundance of 58.78%), reported for its anti-cancerous activity [37], and 3,7,11,15-Tetramethyl-2-hexadec (with a percentage abundance of 11.81%), which has been linked to anticancer and antibacterial activities [38]. To provide similar results, our study was mainly created to evaluate the cytotoxic potential of plant extract with that of conventional medications. Several preliminary studies were performed to confirm the antioxidant activity of *Fagonia arabica* leaf extract (FALE) and assess its potential for cytotoxicity. A practical method for assessing test sample antioxidant activity *in vitro* is cytotoxicity analysis. Using Curcumin as a conventional medication, we got equivalent outcomes in our trial. A study [39] determined that 100 µg/ ml was the highest effective dose, which was inconsistent with the IC_{50} value of 181 µg/ml obtained from the MTT experiment for FALE. On the other hand, the Curcumin MTT assay produced an IC_{50} value of 13.7 µg/ ml, which aligns the reported findings [40]. Furthermore, the FALE Acid Phosphatase Assay gave an IC_{50} value of 109 µg/ ml, which has not been published because no assay of this kind has been carried out with *Fagonia arabica*. The findings [41] associated with the Acid Phosphatase Assay for Curcumin, which showed an IC_{50} value of 11.5 µg / ml.

qPCR was utilized to examine the fold expression of caspase-1, -3, -7, and -9, as well as the Wnt-3a and β-catenin genes by using FALE at 100 µg/ ml and 300 µg/ ml. Our findings showed that caspases were upregulated while the Wnt-3a and β-catenin genes were downregulated in a statistically significant way ($P < 0.001$). These results line up with comparable published research. Researcher showed that Casp-1 performs a caspase-1-dependent process of cell death triggered by microbial infections known as pyroapoptois. Xanthoangelol (XAG), isolated from *Angelica keiskei*, has shown a drug-dependent increase in Casp-1 activity in Hep-G 2 cell lines [42]. The effect of XAG on Casp-1-dependent pyroptosis, indicating the therapeutic potential of natural compounds [43]. Furthermore, the real-time PCR study of *Zea mays* cotton silk extract, discovered a substantial elevation in the fold expression

of Casp-3 and -9, showing enhanced apoptosis ($P < 0.05$) [44]. Emodin, an anthraquinone derivative separated from *Rheum palmatum L.*, a Chinese herb, caused a significant ($P < 0.05$) increase in Caspase-9 and -3 expression in a dose-dependent manner, causing apoptosis through death receptor and mitochondrial pathways [45]. Comparing to control samples, Emodin treatment significantly increased Caspase-3 and -9-fold expression ($P < 0.001$), with decreased expression of Wnt-3a and β-catenin cancer cell lines. Another investigation for the function of miR-195 in colon cancer revealed significant results for both cell viability and β-catenin levels [46]. Doxorubicin treatment proved cytotoxic to MCF-7 cells, to reduce the dosage and its adverse effects, resveratrol was investigated on enhancing the sensitivity of MCF-7 breast cancer cells to doxorubicin [47]. These compounds are receiving more attention in the drug development process due to their anticancer qualities. Here, we found that *Fagonia arabica* significantly downregulated β-catenin and Wnt-3a in MCF-7 cells.

Conclusion

The leaves of *Fagonia arabica* are abundant in polyphenols according to phytochemical studies. Strong antioxidant activity was shown by the greatest amounts of gallic acid and rutin in the methanolic fraction. FTIR and GC-MS spectra provided objective evidence of the diverse chemical constituents present in this complex system, validating its traditional and herbal medicinal uses. Further research may uncover new bioactive compounds in *Fagonia arabica*. *Fagonia arabica* could be a promising natural source of antioxidants for breast cancer treatment. Our study demonstrated the upregulation of caspase-1, -3, -7, and -9, the proapoptotic marker genes, and the downregulation of Wnt-3a and β-catenin, indicating apoptotic cell death in MCF-7 cells. Therefore, *Fagonia arabica* holds potential as a foundation for developing novel lead structures in cancer drug strategies. However, further assessment of its bioactive compounds using techniques such as HPLC and NMR and antioxidant activities in animal models is warranted. Rigorous, well-designed clinical trials are essential to validate the efficacy and safety of natural products in treating breast cancer. For more conclusive proof, future studies should concentrate on large-scale experiments. Our findings may help treat breast cancer with additional validation.

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Conflict of interest: The authors declare no conflict of interest.

Data availability: All data is presented in the manuscript.

Ethics approval: The study was approved by the Institutional Review Board (or Ethics Committee) of the Department of Biological Sciences, International Islamic University, Islamabad 44000, Pakistan, and was given an ethical approval number: No. IIUI/ORIC/Bioethics/110-81. The MCF-7 human breast cancer cell lines used in this study were obtained from the European Collection of Authenticated Cell Cultures (ECACC).

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