separations

MDPI

Article

A Preliminary Cytotoxicity Study of *Fagonia arabica* against Breast (MCF-7), Oral (KB-3-1), and Lung Cancer (A-549) Cell Lines: A Study Supported by Molecular Marker Analysis Using Dual Staining Dyes

[1] Department of Clinical Pharmacy, College of Pharmacy, Najran University, Najran 64462, Saudi Arabia
[2] Department of Pharmacology, College of Pharmacy, Najran University, Najran 64462, Saudi Arabia
[3] Department of Pharmacognosy, College of Pharmacy, Najran University, Najran 64462, Saudi Arabia
[4] Department of Pre-Clinical Research and Drug Development, Cytxon Biosolutions Pvt. Ltd., Hubballi 580031, Karnataka, India
[5] Department of Bioinformatics and Biotechnology, Karnataka State Akkamahadevi Women's University, Vijayapura 586102, Karnataka, India
* Correspondence: dr.aliresearch19@gmail.com; Tel.: +966-553600660

Abstract: Aim: The objective of this research is to present a phytochemical profile of *Fagonia arabica* and to investigate the cytotoxic potential of its extracts against breast, oral, and lung cancer cell lines using MTT assay and dual staining-based mechanistic analysis. Methods: The progressive extraction of *F. arabica* was carried out using the Soxhlet extraction technique. The total phenolic and flavonoid content was calculated as part of the phytochemical profiling performed using GCMS and LCMS methods. The MTT assay was utilized to assess the cytotoxicity against normal L929 cells, as well as malignant A549, MCF-7, and KB-3-1cell lines. Results: The phenolic compounds and flavonoids were the two main elements of the *F. arabica* methanolic extract, with 1323 µg GAE/g of dry weight and 523.07 µg QE/g of dry weight, respectively. The presence of the functional phytochemicals was verified by GCMS and LCMS analyses. Toxicity testing on the L929 cell line found that the *F. arabica* methanol extract was the least harmful, with the highest IC_{50} (296.11 µg/mL). The MTT assay for cell viability against MCF-7 and KB-3-1 yielded significant results, with IC_{50} values of 135.02 µg/mL and 195.21 µg/mL, respectively. The aqueous extract exhibited significant cytotoxicity against the A549 cell lines (IC_{50} 116.06 µg/mL). The molecular marker analyses using dual staining revealed that the methanolic extract successfully triggered apoptosis in the different cancer cells tested. Conclusion: The present data suggest that the methanol extract of *F. arabica* has substantial cytotoxic action against lung, breast, and oral cancer cell lines. Thus, *F. Arabica* would be a promising source of anticancer medicines, warranting more research to identify the lead molecules with anticancer properties.

Keywords: *Fagonia arabica*; breast cancer; oral cancer; lung cancer; apoptosis; MTT; dual staining assay

Citation: Walbi, I.A.; Alshabi, A.M.; Alkahtani, S.A.; Shaikh, I.A.; Abdel-Wahab, B.A.; Khateeb, M.M.; Habeeb, M.S.; Orabi, M.A.A.; Shettar, A.K.; Hoskeri, J.H. A Preliminary Cytotoxicity Study of *Fagonia arabica* against Breast (MCF-7), Oral (KB-3-1), and Lung Cancer (A-549) Cell Lines: A Study Supported by Molecular Marker Analysis Using Dual Staining Dyes. *Separations* **2023**, *10*, 110. https://doi.org/10.3390/separations10020110

Academic Editors: Norizan Ahmat, Juriyati Jalil and Victoria Samanidou

Received: 10 December 2022
Revised: 21 January 2023
Accepted: 31 January 2023
Published: 3 February 2023

1. Introduction

Cancer is the second most significant cause of death worldwide, causing about 9.6 million deaths [1]. The cancer rate has been continuously advancing for decades. There was a 0.54 percent incidence of cancer worldwide in 1990, but in 2017, that number climbed to 1.3%. Nearly 1.5 million individuals every year lose their lives to lung cancer [2], whereas breast cancer is the most common malignant disease affecting women [3]. Mouth and oral cancers include larynx, hypopharynx, oropharynx, and lip cancers. Lip and oral cavity cancers are common, with about 377,700 occurrences worldwide in 2020. In Saudi Arabia, the mean age-standardized rate of oral cancer is 2.9 per 100,000 people; it was 1.5 for females and 1.4 for males [4]. Cancer prevention and treatment have been improving in recent years. Cancer patients have a dismal survival rate despite chemotherapy, radiation,

and targeted therapy [5–9]. Saudi Arabia ranks second in the Gulf countries for cancer mortality rates (62.78%) [10].

Oral cancer has a higher incidence than any other form of head and neck cancer. Although it is one of the most common types of cancer, survival rates have not improved significantly in recent years despite significant strides in both research and treatment. Human papillomavirus (HPV) infection, heavy alcohol use, and smoking all contribute to oral cancer development. Symptoms typically include a persistent mouth sore, a lump, or a white or red spot inside the mouth [11].

Lung and breast cancer are among the top three major malignancies. Many people with lung cancer experience weight loss, fatigue, bloody coughs, and difficulty swallowing [12]. Traditional lung cancer treatments include chemotherapy, immunotherapy, radiation, and surgery. Chemotherapy side effects such as neurotoxicity, alopecia, tiredness, and mouth ulcers worry specialists [13–15].

As a result, scientists are focusing on plants and plant-based products to discover effective medications to combat cancer. A wide range of plant species has been found to have therapeutic effects in treating and preventing illness [16]. Alternative cancer treatments based on plant-derived substances have been developed [17]. It has been shown that plant-based anticancer compounds presented fewer side effects compared to many conventional chemotherapeutics [18,19].

The *Fagonia* is a genus of wild flowering plants (Zygophyllaceae) distributed throughout Africa, the Mediterranean Basin, the Middle East, and India. *Fagonia arabica* is known as Virgin's Mantle in English, Fagonie in German, and Shawka al-Baidaa in Arabic. *F. arabica* is rich in secondary metabolites such as glycosides, flavonoids, triterpenes, saponins, and steroids [20].

Various species of the genus *Fagonia* have been known to possess potent pharmacological activities, i.e., anti-inflammatory [21], anti-allergic [22], neuroprotective [23], androgenic, endocrinological, antimicrobial, and cytotoxic activities [24–26]. *F. arabica* is reported to be used in stomatitis, blood, and hormonal disorders [26]. The plant's dried leaves and stems have diuretic properties [26,27], purifying the blood and possessing de obstruent properties [28]. The plant also treats skin disorders, smallpox, and tumors [29,30].

Antioxidant, analgesic, anti-inflammatory, and antipyretic properties in *F. arabica* have been linked to its phenolic content [31]. Moreover, it helps revive antioxidant enzymatic activity and reduce lipid peroxidation [32].

There have been reports of several species from the *Fagonia* genus having cytotoxic effects on breast cancer (MCF-7), liver cancer (HepG-2), and lung cancer (A549). Nevertheless, even though *F. arabica* is abundant in polyphenolic chemicals, saponins, and flavonoids, little is known regarding its cytotoxic potential.

Hence, we thought it worthwhile to investigate the cytotoxic potential in breast, lung, and oral cancer cell lines and study the underlying molecular mechanisms of its cytotoxic effect. To examine its cytotoxicity, we employed an MTT assay, and an acridine orange/ethidium bromide (AO/EB) dual staining experiment was used to investigate the molecular processes. The MTT assay is widely used because it provides a quantitative measure of cellular metabolic activity, which is a good predictor for cell survival, division, and cytotoxic potential [33]. The AO/EB strategy is effective because AO can enter all cells through diffusion, whereas EB cannot breach a cell membrane except in necrotic or dead cells. The percentage of apoptotic cancer cells detected by dual AO/EB labeling was not substantially different from that detected by flow cytometry ($p > 0.05$), according to research conducted by Liu et al., Shanghai, China, in 2015. Thus, dual AO/EB labeling provides a more valuable and convenient approach than flow cytometry for identifying apoptosis in cancer cells and assessing tumor chemosensitivity [34]. The aim of the current research was to determine the phytochemical composition of an indigenously grown *F. arabica* plant (collected from the Najran University Campus, Southern Saudi Arabia), as well as to determine the cytotoxic effects of its various extracts on non-cancerous (L929), cancerous

breast cancer (MCF7), oral cancer (KB-3-1), and lung cancer (A549) cell lines. Also explored were its molecular pathways for inducing apoptosis.

2. Methodology

2.1. Plant Collection, Extraction, and Phytochemical Analysis

Whole plants of the *F. arabica* were gathered from the premises of Najran University, Saudi Arabia, and identified and confirmed by an expert pharmacognosist, Prof. Mohamed A. A. Orabi, Department of Pharmacognosy, Faculty of Pharmacy, Najran University, Najran, Saudi Arabia, and a herbarium specimen (Fag.1503–2021) was preserved. After collecting disease-free plant materials, they were cleaned with running tap water to remove dust particles and dried with blotting paper before being rinsed with alcohol to remove any bacteria or microbial pathogens. The plant material was dried in the shade at room temperature (26–27 °C) and crushed into a coarse powder with the help of a mechanical method using a grinder. The whole plant material, including the roots and the aerials parts, was used for extraction since, in traditional medicine, whole plant extracts are utilized compared to individual isolated phytochemicals. Evidence suggests that crude plant extracts often demonstrate more potent in vitro and in vivo activity than purified phytochemicals at comparable doses. Positive interactions between the ingredients of whole plant extracts may illustrate why crude extracts are frequently more effective.

For the Soxhlet extraction, we used a solvent ratio of 1:10. For each cycle, approximately 100 g of dried *F. arabica* powder was used. In a sequence of ascending polarity, hexane, ethyl acetate, methanol, and distilled water were utilized in the solvent extraction procedure. In the case of each solvent in the Soxhlet apparatus, the temperature of the heating mantle was changed according to the solvent used for the extraction: for hexane, it was 68 °C; for ethyl acetate, 55 °C; for methanol, 50 °C and distilled water, 70 °C. Each solvent was subjected to 12 to 24 h of extraction. Before proceeding with the extraction process using different solvents, the plant material that had been previously extracted was always dried. Using a rotary vacuum evaporator (Rota evaporator, butchi@300), the extracts were subsequently concentrated. The concentrated extracts were then dried in desiccators to provide a final yield of hexane (2.1%), ethyl acetate (2.7%), methanol (3.6%), and aqueous (3.1%), which were stored in an airtight container at 4 °C for future use. The formula for percentage yield was:

$$\% \text{ Yield} = R/S \times 100,$$ where R = extracted weight of extract residue, and S = initial sample weight.

2.2. Chemicals and Instrumentation

The cell lines L929, A549, KB-3-1, and MCF7 came from the National Centre for Cell Science (NCCS), situated in Pune, India. The Sigma-Aldrich company, St. Louis, Missouri, USA, provided the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), which had a purity of 97.5%. All remaining chemicals were of analytical quality and acquired from HiMedia, Mumbai, India. The following instruments were used in this study: Rota evaporator, Buchi-R300, Flawil, Switzerland; Inverted Microscope, Magnus Magvision software, New Delhi, India; Elisa reader, Biobase, Shandong, China; GC-MS model GCMS-QP2010S, Shimadzu, Kyoto, Japan; LC-MS-8040, Shimadzu, Kyoto, Japan.

2.3. Phytochemical Analysis

In this study, a preliminary phytochemical screening of the sequential extracts of *F. arabica* was conducted to investigate a variety of secondary metabolites [35].

2.4. Estimation of Total Phenols and Flavonoid Content

Spectrophotometry was used to determine the total phenolic component content of the plant extract, as was previously published [36]. Calibration curves for gallic acid (GA) were constructed at 20–100 ng/mL. Finally, the extract's phenolic contents were standardized to

gallic acid equivalents (GAEs), expressed as mg GAE/g of dry weight (dw). The overall phenol concentration was calculated using the calibration curve (y = 0.001x + 0.113).

A colorimetric assay with the aluminum chloride technique was used to determine the total flavonoid concentration [37]. We developed standards for quercetin concentrations ranging from 20 to 100 µg/mL. One mL of every quercetin concentration was put into a 10 mL volumetric flask containing four mL of double-distilled water in order to measure the quercetin concentrations in the methanol. At time t = 0, three chemicals were added: sodium nitrite 5% (0.3 mL), aluminum chloride 10% (0.3 mL), and sodium hydroxide 1M (2 mL). The liquid was ready for use after 2.4 mL of double-distilled water was swiftly added. At 510 nm, the absorbance of the pink color mixture was measured in comparison to a blank without quercetin. The calibration curve was created using the normal quercetin absorbance values. Quercetin equivalents (y = 0.265 × 0.152) were used to express the flavonoid concentrations as mg QE/g dry weight.

2.5. GCMS Analysis

As was previously stated [38], GC-MS analysis was carried out on a series of solvent extracts of *F. arabica*. The analysis was conducted using a GC-MS instrument (model GCMS-QP2010S) equipped with a fused silica column. Helium was used as the carrier gas at a constant flow rate of 1 mL/min to accomplish the separation. Through a split injector, 1 µL of *F. arabica* methanol extract was injected into the apparatus. The temperature of the column was set to 80 °C initially, while the temperature of the injector was set to 260 °C. Throughout the process, the temperature flow was set to increase by 10 °C per minute, with the following parameters: column flow: 1.00 mL/min, pressure: 65.0 kPa, linear velocity: 36.8 cm/s, total flow: 24.0 mL/min, and purge flow: 3.0 mL/min. Comparisons of relative retention time and mass spectra with the standard compounds validated the identification of the main components of the plant extract.

2.6. Liquid Chromatography–Mass Spectrometry (LC-MS) Analysis

Using a binary pump and an LC-MS-8040 (Shimadzu), we analyzed the chemical components of an *F. arabica* methanol fraction. An ESI-equipped mass spectrometer was connected to the HPLC. For this particular analysis, a C18 HPLC column was utilized. The total flow rate of the solvent was 0.2 mL/min, and the solvent was methanol in a water ratio of 80:20. The direct injection mode with a positively biased Electron Spray Ionization (ESI) probe was used for the detection. The sample flow rate was 8 µL/min, and the capillary temperature was maintained at 280 °C. Altogether, 3 µL of the sample was injected for the analysis [39].

2.7. In Vitro Cytotoxicity of F. arabica Extracts

The MTT assay was used to evaluate the cytotoxicity of various *F. arabica* solvent extracts, as previously mentioned [40]. In summary, 5 mg/mL of an MTT solution (Sigma-Aldrich, St. Louis, MI, USA) was dissolved in a PBS. Direct addition of 20 µL of 5 mg/mL MTT solutions was made to each required well. Non-cancerous L929, breast cancer MCF-7, lung cancer A549, and oral cancer (KB-3-1) cell lines were used in the current study. In 96-well plates, cells were initially plated at a density of 1×10^5 cells per mL. Following a 24-h incubation period at 37 °C, cells were treated with 50, 100, 150, 200, and 250 µg/mL of various fractions. Cells that were not treated at all served as a negative control, while those treated with the gold standard medication cisplatin were used as a positive control. After incubating at 37 °C for 4 h, the MTT solution was added to each well. At 570 nm, an ELISA reader (Bio-Tek Instruments, Winooski, VT, USA) was employed to measure the optical density. Assays were performed in triplicate for each concentration. These IC_{50} values were calculated by plotting a linear regression curve.

$$\text{Inhibition Percentage} = (\text{OD of test sample}/\text{OD of control}) \times 100$$

2.8. Detection of Live and Dead Cells Using Acridine Orange/Ethidium Bromide Dual Staining

All three cancer cells were cultivated onto 24-well plates with a counting cell density of 20,000 cells/well and incubated for 24 h at 37 °C and 5% CO_2 conditions. Additionally, the developed cells were exposed to *F. arabica* at its IC_{50} concentration. Additionally, after 24 h, the used media was withdrawn from the wells, and cells were fixed with cooled methanol for 30 min at room temperature. Acridine orange and ethidium bromide were mixed 1:1 and stained for 15 min at 37 °C in the dark. After the stain was removed, cells were given two PBS washes, and 1 mL of the PBS was added on top. Using a fluorescence imager at 20× magnification, cells were captured in the green and red bands [41].

2.9. Statistical Analysis

A one-way ANOVA followed by a Dunnett's test were conducted in GraphPad Prism 6 to evaluate statistical significance ($p < 0.05$). The standard deviation and mean were determined by conducting 3 different experiments (n = 3).

3. Results

3.1. Qualitative Phytochemical Analysis of F. arabica

The results indicated the existence of glycosides, flavonoids, terpenoids, steroids, and saponins in all the tested extracts. However, the methanol and aqueous extracts showed the existence of flavonoids, phenols, and terpenoids (Table 1).

Table 1. Phytochemical analysis of different solvent extracts of *F. arabica*.

Tests	Hexane	Ethyl Acetate	Methanol	Aqueous
Alkaloids	−	−	−	−
Flavonoids	−	−	+	+
Glycosides	−	+	−	−
Phenols	−	−	+	+
Saponins	−	+	−	−
Tannins	−	−	−	−
Terpenoids	−	−	+	+
Steroids	+	+	−	−

"+" means "present"; "−" means "absent".

3.2. Quantification of Total Phenols and Flavonoids in F. arabica

According to the phytochemical analysis, a total phenol content quantification study was performed on both the methanol and aqueous extract of *F. arabica*. According to the quantitative findings, the phenolic content of the methanol extract was greater than that of the aqueous extract, i.e., 1323 µg GAE/g, whereas the aqueous extract exhibited 874 µg GAE/g. In the case of the total flavonoid content, the methanol extract showed 523.07 µg/g, whereas the aqueous extract showed 279.18 µg/g.

3.3. Cytotoxicity of F. arabica Extracts In Vitro

The present investigation used an MTT test to evaluate the efficacy of various *F. arabica* solvent extracts. The percentage of cell viability in the non-cancerous (L929) cell line reduced as the concentration of the extracts was increased. At higher doses, the toxic character of nearly all of the extracts examined became apparent. Hexane, ethyl acetate, methanol, and aqueous extracts all significantly decreased the cell viability when used at increasing doses (Table 2), whereas the morphological effects due to each extract are depicted in Figure S1 (Supplementary Data).

Using an MTT cell viability assay and cisplatin as a reference drug, the extracts were tested for cytotoxicity against the cancerous cell lines. The solvent extracts inhibited the cell growth and cell survival in the tested cell lines in a concentration- and time-dependent manner, with the effect being statistically significant ($p < 0.001$). Lung cancer A549 cells

were most effectively inhibited by all the extracts tested, and this effect was dose-dependent (Table 3), in the breast cancer MCF7 (Table 4), and oral cancer KB-3-1 (Table 5) cell lines. In comparison, the selected solvent extracts showed effective activity against breast cancer compared to the lung and oral cancer cell lines. The morphological effects of each solvent extract for A549, MCF-7, and KB-3-1 are shown in Figures S2–S4, respectively. The cell viability data and first observations showed that the methanol extract was significantly more effective than the other extracts examined.

Table 2. Cytotoxicity of different solvent extracts of *F. arabica* against L929 cell line.

Concentration (µg/mL)	Percentage (%) of Cell Viability			
	Hexane	Ethyl Acetate	Methanol Extract	Aqueous Extract
50	90.85 ± 0.014 *	90.85 ± 0.009 *	94.36 ± 0.002	78.09 ± 0.005 *
100	77.87 ± 0.016 *	72.28 ± 0.013 *	85.22 ± 0.002 *	70.71 ± 0.016 *
150	67.86 ± 0.001 *	58.75 ± 0.008 *	77.22 ± 0.005 *	55.42 ± 0.021 *
200	47.42 ± 0.001 *	45.66 ± 0.007 *	65.30 ± 0.022 *	49.25 ± 0.003 *
250	32.80 ± 0.015 *	28.11 ± 0.010 *	50.15 ± 0.022 *	33.16 ± 0.018 *

The data are presented as mean SEM (n = 3), with significance determined at $p < 0.05$. Dunnett's test was used to compare the means using a one-way ANOVA. * $p < 0.001$ compared to the control group (100% cell viability).

Table 3. Anticancer activity of *F. arabica* extracts against lung cancer A549 cell line.

Concentration in µg/mL	Percentage (%) of Cell Viability			
	Hexane	Ethyl Acetate	Methanol	Aqueous
50	72.32 ± 0.005 *	67.04 ± 0.011 *	80.13 ± 0.014 *	76.59 ± 0.012 *
100	62.90 ± 0.012 *	60.74 ± 0.002 *	73.70 ± 0.001 *	51.74 ± 0.015 *
150	45.73 ± 0.010 *	51.86 ± 0.002 *	65.30 ± 0.015 *	39.19 ± 0.015 *
200	27.31 ± 0.011 *	42.67 ± 0.009 *	57.74 ± 0.008 *	19.38 ± 0.003 *
250	10.86 ± 0.001 *	33.43 ± 0.011 *	50.96 ± 0.014 *	8.36 ± 0.002 *

The data are presented as mean SEM (n = 3), with significance determined at $p < 0.05$. Dunnett's test was used to compare the means using a one-way ANOVA. * $p < 0.001$ compared to the control group (100% cell viability).

Table 4. Anticancer activity of *F. arabica* extracts against breast cancer MCF-7 cell line.

Concentration in µg/mL	Percentage (%) of Cell Viability			
	Hexane	Ethyl Acetate	Methanol	Aqueous
50	83.22 ± 0.008 *	70.71 ± 0.012 *	65.24 ± 0.003 *	81.09 ± 0.009 *
100	77.15 ± 0.007 *	63.33 ± 0.009 *	59.94 ± 0.021 *	58.46 ± 0.007 *
150	65.68 ± 0.009 *	46.44 ± 0.006 *	46.22 ± 0.002 *	51.53 ± 0.003 *
200	55.95 ± 0.012 *	32.73 ± 0.008 *	37.54 ± 0.012 *	38.41 ± 0.009 *
250	49.01 ± 0.004 *	25.19 ± 0.002 *	25.90 ± 0.022 *	23.55 ± 0.014 *

The data are presented as mean SEM (n = 3), with significance determined at $p < 0.05$. Dunnett's test was used to compare the means using a one-way ANOVA. * $p < 0.001$ compared to the control group (100% cell viability).

Table 5. Anticancer activity of *F. arabica* extracts against oral cancer KB-3-1 cell line.

Concentration (µg/mL)	Percentage (%) of Cell Viability			
	Hexane	Ethyl Acetate	Methanol	Aqueous
50	93.26 ± 0.013	94.74 ± 0.035	85.22 ± 0.015 *	93.43 ± 0.004
100	81.27 ± 0.007 *	79.17 ± 0.004 *	76.43 ± 0.002 *	83.50 ± 0.028 *
150	72.22 ± 0.022 *	70.93 ± 0.023 *	63.98 ± 0.005 *	74.86 ± 0.039 *
200	59.23 ± 0.030 *	56.54 ± 0.003 *	46.33 ± 0.014 *	69.50 ± 0.032 *
250	48.99 ± 0.017 *	45.79 ± 0.033 *	36.08 ± 0.010 *	54.29 ± 0.007 *

The significance level was set at $p < 0.05$, and the data are provided as mean SEM (n = 3). Using a one-way ANOVA, the means were compared using Dunnett's test; * $p < 0.001$ compared to the control group (100% cell viability).

The morphological analysis is likewise consistent with the MTT findings. Changes in cellular morphology were seen between the untreated MCF-7, A549, and KB-3-1 cell lines and the test samples. The cells in the untreated group had a more distinct shape and were more numerous, without intracellular gaps. In contrast, when the test drug concentration grew, the cell number dropped, and intracellular gaps were observed in the test sample-treated cells (Figures S2–S4). This was in addition to the presence of other morphological abnormalities, such as cell shrinkage, apoptotic bodies, cell turgidity, and membrane blabbing. These characteristics are typical of cells that have begun the apoptotic process. Microscopical and MTT testing results indicate that *F. arabica* has the potential to cause apoptosis in the breast cancer MCF-7 cell line, the lung cancer A549 cell line, and the oral cancer KB-3-1 cell line.

In addition, the IC_{50} value for each of the treated cell lines was determined using the standard calibration curve. The IC_{50} details for all the extracts and standard cisplatin are depicted in Table 6.

Table 6. IC_{50} value (µg/mL) of *F. arabica* extracts against different cell lines.

Extract/Standard	L929	MCF-7	A549	KB-3-1
Hexane	195.72	240.47	130.55	244.93
Ethyl acetate	180.04	140.46	156.74	212.98
Methanol	296.11	135.02	254.79	195.21
Aqueous	182.89	152.24	116.06	286.17
Cisplatin	10.54	2.87	12.82	16.27

3.4. Acridine Orange/Ethidium Bromide Dual Staining Analysis

In the present study, the IC_{50} concentration of *F. arabica* was treated on the lung cancer A549 and breast cancer MCF-7 cell lines and subjected to the dual staining analysis. The results revealed that compared to the MTT results, even in the dual staining assay, the *F. arabica* extract exhibited significant activity in the breast cancer MCF-7 cell line with more apoptotic or dead cells. The results were compared to the standard drug, cisplatin, and the untreated cells were taken as a negative control. In the case of the negative group, all the cells in the tested cancer cell lines appeared as viable cells (the green color). In contrast, in the standard drug cisplatin-treated cell lines, more dead cells and fewer viable cells were observed (Figures 1 and 2).

3.5. GCMS and LCMS Analysis

The methanol extract came out as a potent extract; hence, the methanol extract of *F. arabica* was subjected to GC-MS analysis to identify the phytochemical compounds. The GC-MS results showed the presence of five sharp peaks in the gas chromatogram. Each peak was subjected to a similarity search using the NIST library database to identify the compounds. Majorly, the GC-MS analysis showed the presence of mome inositol, neophytadiene, methylpalmitate, methyl octadeca-9,12-dienoate, and emery oleic acid ester as the major compounds. These compounds are known for several applications, such as industrial applications, organic synthesis, and biological activities (Table 7), and the GCMS spectrum of the compounds is shown in Figure S5.

Pertaining to the LC-MS analysis of methanol extract, the results revealed that the methanol extract showed the presence of 10b-Methyl-3,4,5,6,10b,11-hexahydro-2H-4b-aza-chrysene-1,12-dione as major compounds. This flavonoid compound is known for good antioxidant and anticancer applications (Table 8 and Figure S6).

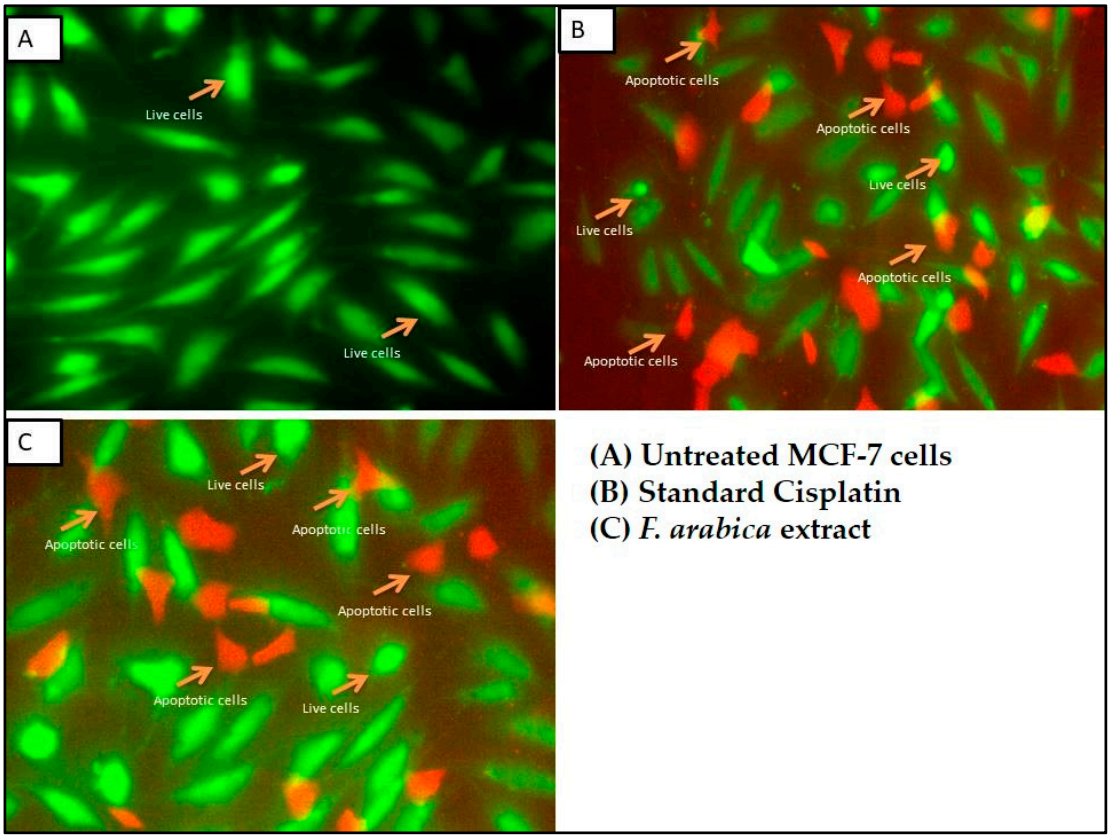


Figure 1. Detection of live and apoptosis cells in treated breast cancer MCF-7 cells.

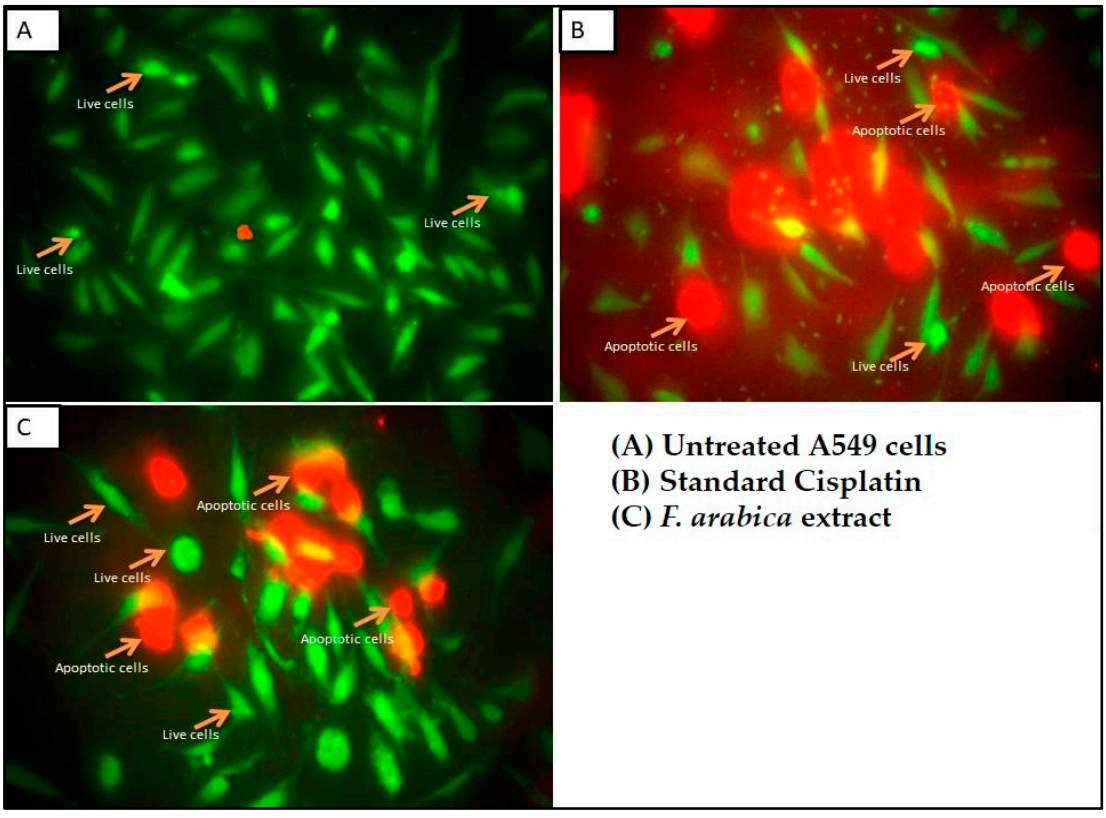
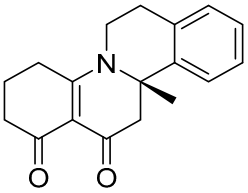


Figure 2. Detection of live and apoptosis cells in treated lung cancer A549 cells.

Table 7. GC-MS-identified compounds present in methanol extract of *F. arabica*.

Peak No.	Compound Name	R_t	Base m/z	Nature	Uses
1	Mome inositol	25.949	87.05	Sugar derivative	Anti-alopecia, anti-cirrhotic, and anti-neuropathic [42,43]
2	Neophytadiene	26.611	68.05	Diterpene	Anti-inflammatory and antimicrobial agent [44]
3	Methylpalmitate	28.374	74.05	Fatty acid methyl ester	Used in detergents, lubricants, and animal feeds [45]; anti-inflammatory and anti-fibrotic agent [46]
4	Methyl octadeca-9,12-dienoate	31.601	67.05	Fatty acid methyl ester	Industry use, antioxidant [47,48]
5	Oleic acid ester	31.703	55.05	Fatty acid methyl ester	Antioxidant, antibacterial, antihypertensive [49,50]

Table 8. LC-MS-based profiling of Methanol extract of *F. arabica*.

Sl.no	Structure	Compound Name	Retention Time	Nature	Uses
1		10b-Methyl-3,4,5,6,10b,11-hexahydro-2H-4b-aza-chrysene-1,12-dione	11.994	Nitrogenous compound	Anticancer, antioxidant [51,52]

4. Discussion

Several anticancer drugs have been discovered to increase therapeutic outcomes in response to the rising cancer prevalence. Despite the constant discovery of new chemotherapeutic medicines, minimizing side effects during therapy is still challenging. For instance, conventional anticancer drugs' nephrotoxicity and immunosuppressive effects during treatment have been observed to be unpredictable [53]. In addition, medication resistance may emerge throughout therapy, requiring greater dosages to reestablish the same anticancer benefits. Various natural plant extracts, including vincristine and paclitaxel, have been investigated and developed as anticancer drugs in current conventional treatments. As a result, we selected *F. arabica* plant extract to investigate its cytotoxic potential against various cancer cell lines; the active components of *F. arabica* were also identified using spectral analysis.

In this study, we tested the cytotoxic efficacy of *F. arabica* extracts. The results showed that the tested methanolic extract was cytotoxic against all the tested cell lines. Within the group of the examined cancer cell lines, *F. arabica* showed the most promise against breast cancer, followed by oral and lung cancer. The MTT cell viability values for the plant extract were reasonable. The results of the MTT assay showed aberrant morphological characteristics, such as apoptotic cells, nuclear condensation, cell turgidity, and membrane blabbing. All of these are characteristics of cells going through apoptosis. The results from the MTT and microscopic studies suggest that the test samples may be causing apoptosis in the cancer cell lines MCF-7, A549, and KB-3-1. The methanol extract of *F. arabica* has demonstrated very encouraging outcomes in breast and oral cancer cell lines, with a substantially lower IC_{50} value among all of the evaluated solvent extracts, according to the overall data. With an IC_{50} value of 296.11 µg/mL, it also demonstrated less toxicity in the tested non-cancerous cell line.

Further studies were carried out on the methanol extract to determine the mechanism and its phytoconstituents using GCMS, LCMS, and dual-staining assays. The GC-MS and LC-MS results showed that multiple secondary metabolites were present. These identified compounds are known to have a wide range of uses, including as flavoring agents, cosmetics, detergents, and building blocks for chemical synthesis. These findings are con-

sistent with the previous literature. For example, Negoumy (1986) discovered six common flavonoids with isorhamnetin and herbacetin glucoside and rutenoside connections [54]. El-Wakil (2007) discovered two distinct phytoconstituents with rhamnoside linkages to kaempferol and acacetin in the water/methanol extract of this plant [55]. Some phenolic components in the plant were shown to have antioxidant action by Khaled Tawaha in 2007 [56].

To maintain a redox balance, the production and elimination of reactive oxygen species (ROS) must be tightly regulated. When things get out of balance, oxidative stress and cellular damage occur due to a redox imbalance, which can lead to several disease states, including cancer. In the mitochondrial respiratory chain and other metabolic activities, oxygen consumption leads to the production of ROS. It plays a role in the development and advancement of cancer. Cell proliferation is just one of many biological processes regulated by reactive oxygen species (ROS), which also affects signaling pathways such as those involving growth factors and mitogenic pathways. This promotes cancerous tumor development and the onset of carcinogenesis by stimulating unchecked cell proliferation [57]. Therefore, antioxidant chemicals that lessen oxidative stress are gaining popularity as a means of combating cancer [58]. According to phytochemical studies of *F. arabica*, it contains a high concentration of phenolic compounds, which are well-known antioxidants [55,59]. Thus, it is assumed that any drug that reduces oxidative stress is useful in treating cancer. It is reasonable to presume that the antioxidant phytochemicals found in *F. arabica* are responsible for the plant's cytotoxic potential against the tested cell lines.

Furthermore, we assessed the mechanism of the cytotoxic action of *F. arabica* by performing a dual-staining assay. Cell death was analyzed using a combination of acridine orange and ethidium bromide labeling. Both ethidium bromide and acridine orange, which are both cell-permeable dyes, attach to the nucleic acid and emit green and red fluorescence, respectively, when the membrane is disrupted. Therefore, the two dyes help identify the cause of cell death [60]. In the current study, the dual staining assay showed that the *F. arabica* extract-treated cancer cell lines had more apoptotic or dead cells compared to the controls. Cisplatin and the untreated cells were used as controls. From the above results, we can assume apoptosis and cell necrosis as possible mechanisms responsible for the cytotoxic potential of *F. arabica*.

Study limitations: There are a few study limitations worth addressing. The MTT assay, which was used as a cytotoxicity assay, has some drawbacks, including poor repeatability, interactions between polyphenolic substances and the MTT, interference between the compounds' absorbance spectra at 570 nm, and DMSO toxicity. For determining cell viability, the DRAQ7 flow cytometry colorimetric test method is preferred and suggested as a substitute for an MTT assay [61].

Study implications: Herbal remedies are rapidly being accepted as effective supplemental cancer treatments. Many clinical studies have reported the favorable effects of herbal medicines on cancer patients' survival, immunological regulation, and quality of life when used in conjunction with conventional cancer therapies. These phytochemicals have been developed into cancer treatments, and new technologies are emerging to advance the field. Nanoparticles for nanomedicines are a novel technology that aims to improve the anticancer effects of compounds produced from plants by managing the compound's release and researching alternative administration techniques. As a result, the current study should provide useful technological support for the evidence-based use of herbal medications in cancer therapy.

5. Conclusions

F. arabica showed a significant cytotoxic effect against oral cancer KB-3-1, breast cancer MCF-7, and lung cancer A549 cell lines. Evidence from dual staining microscopy suggests that the tested drug induced apoptosis in the tested cell lines through a sequential molecular mechanism, which can be attributed to DNA damage and necrosis. Furthermore, *F. arabica* has strong cytotoxic activity, comparable to that of the gold standard medication, cisplatin.

Since *F. arabica* shows promise as a source of anticarcinogenic medicines, it should be investigated further to find lead molecules with potential as cancer chemotherapies. Additional research is needed before the results of this study can be applied to clinical testing.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations10020110/s1, Figure S1: Cytotoxicity of *F. arabica* against noncancerous L929 cell line. Cisplatin was used as a standard drug; Figure S2: Cytotoxic activity of *F. arabica* against Lung cancer A549 cell line. Cisplatin was used as a standard drug; Figure S3: Cytotoxic activity of *F. arabica* against Breast cancer MCF-7 cell line. Cisplatin was used as a standard drug. Figure S4: Cytotoxic activity of *F. arabica* against Breast cancer MCF-7 cell line. Cisplatin was used as a standard drug; Figure S5: GC chromatogram and mass spectra of methanol extract of *F. arabica*; Figure S6: LCMS chromatogram and mass spectra of methanol extract of *F. arabica*.

Author Contributions: Conceptualization, A.M.A., M.A.A.O., A.K.S. and B.A.A.-W.; Funding acquisition, A.M.A.; Investigation, I.A.S., M.M.K. and A.K.S.; Methodology, M.A.A.O., B.A.A.-W. and A.K.S.; Project administration, S.A.A.; Resources, A.M.A. and I.A.W.; Software, I.A.S. and M.M.K.; Supervision, M.A.A.O., A.M.A. and I.A.W.; Visualization, S.A.A.; Writing—original draft, I.A.S., M.S.H. and A.K.S.; Writing—review & editing, I.A.S., M.A.A.O., A.K.S. and J.H.H. All authors have read and agreed to the published version of the manuscript.

Funding: The authors would like to acknowledge the support of the Deputy for Research and Innovation, Ministry of Education, Kingdom of Saudi Arabia, for a grant (NU/IFC/ENT/01/006) under the Institutional Funding Committee at Najran University, Kingdom of Saudi Arabia.

Data Availability Statement: All data has been included in the manuscript.

Acknowledgments: The authors would like to acknowledge the support of the Deputy for Research and Innovation, Ministry of Education, Kingdom of Saudi Arabia, for a grant (NU/IFC/ENT/01/006) under the Institutional Funding Committee at Najran University, Kingdom of Saudi Arabia.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer Statistics, 2020. *CA Cancer J. Clin.* **2020**, *70*, 7–30. [CrossRef] [PubMed]
2. Abdul Rahman, J. Lung Cancer in Saudi Arabia. *J. Thorac. Oncol.* **2019**, *14*, 957–962.
3. Breast Cancer Statistics. *J. Natl. Cancer Inst.* **2000**, *92*, 445. [CrossRef] [PubMed]
4. Alshehri, B.M. Trends in the Incidence of Oral Cancer in Saudi Arabia from 1994 to 2015. *World J. Surg. Oncol.* **2020**, *18*, 217. [CrossRef]
5. Eckhardt, B.L.; Francis, P.A.; Parker, B.S.; Anderson, R.L. Strategies for the Discovery and Devel-opment of Therapies for Metastatic Breast Cancer. *Nat. Rev. Drug Discov.* **2012**, *11*, 479–497. [CrossRef]
6. Cao, H.; Zhang, Z.; Zhao, S.; He, X.; Yu, H.; Yin, Q.; Zhang, Z.; Gu, W.; Chen, L.; Li, Y. Hydropho-bic Interaction Mediating Self-Assembled Nanoparticles of Succinobucol Suppress Lung Metastasis of Breast Cancer by Inhibition of VCAM-1 Expression. *J. Control. Release* **2015**, *205*, 162–171. [CrossRef]
7. Gennari, A.; Conte, P.; Rosso, R.; Orlandini, C.; Bruzzi, P. Survival of Metastatic Breast Carcinoma Patients over a 20-Year Period: A Retrospective Analysis Based on Individual Patient Data from Six Consecutive Studies: A Retrospective Analysis Based on Individual Patient Data from Six Consecutive Studies. *Cancer* **2005**, *104*, 1742–1750. [CrossRef]
8. Dan, Z.; Cao, H.; He, X.; Zhang, Z.; Zou, L.; Zeng, L.; Xu, Y.; Yin, Q.; Xu, M.; Zhong, D.; et al. A PH-Responsive Host-Guest Nanosystem Loading Succinobucol Suppresses Lung Metastasis of Breast Cancer. *Theranostics* **2016**, *6*, 435–445. [CrossRef]
9. Long, Q.; Wang, Y.; Che, G. Primary Lung Cancer after Treatment for Breast Cancer. *Int. J. Womens Health* **2021**, *13*, 1217–1225. [CrossRef]
10. Alqahtani, W.S.; Almufareh, N.A.; Domiaty, D.M.; Albasher, G.; Alduwish, M.A.; Alkhalaf, H.; Al-muzzaini, B.; Al-Marshidy, S.S.; Alfraihi, R.; Elasbali, A.M.; et al. Epidemiology of Cancer in Saudi Arabia Thru 2010–2019: A Systematic Review with Constrained Meta-Analysis. *AIMS Public Health* **2020**, *7*, 679–696. [CrossRef]
11. Mouth Cancer. Available online: https://www.mayoclinic.org/diseases-conditions/mouth-cancer/symptoms-causes/syc-20350997 (accessed on 9 December 2022).
12. Polanski, J.; Jankowska-Polanska, B.; Rosinczuk, J.; Chabowski, M.; Szymanska-Chabowska, A. Qual-ity of Life of Patients with Lung Cancer. *Onco. Targets. Ther.* **2016**, *9*, 1023–1028. [CrossRef] [PubMed]
13. Chemotherapy Side Effects. Available online: https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/chemotherapy/chemotherapy-side-effects.html (accessed on 9 December 2022).

14. Le Saux, O.; Ray-Coquard, I.; Labidi-Galy, S.I. Challenges for Immunotherapy for the Treatment of Platinum Resistant Ovarian Cancer. *Semin. Cancer Biol.* **2021**, *77*, 127–143. [CrossRef] [PubMed]
15. Pucci, C.; Martinelli, C.; Ciofani, G. Innovative Approaches for Cancer Treatment: Current Perspec-tives and New Challenges. *Ecancermedicalscience* **2019**, *13*, 961. [CrossRef]
16. Wang, H.; Khor, T.O.; Shu, L.; Su, Z.; Fuentes, F.; Lee, J.H.; Kong, A.N.T. Plants Against Cancer: A Review on Natural Phytochemicals in Preventing and Treating Cancers and Their Druggability. *Anticancer Agents Med. Chem.* **2012**, *12*, 1281–1305. [CrossRef] [PubMed]
17. Gutheil, W.G.; Reed, G.; Ray, A.; Anant, S.; Dhar, A. Crocetin: An Agent Derived from Saffron for Prevention and Therapy for Cancer. *Curr. Pharm. Biotechnol.* **2012**, *13*, 173–179. [CrossRef]
18. Ahmad, R.; Ahmad, N.; Naqvi, A.A.; Shehzad, A.; Al-Ghamdi, M.S. Role of Traditional Islamic and Arabic Plants in Cancer Therapy. *J. Tradit. Complement. Med.* **2017**, *7*, 195–204. [CrossRef]
19. Iqbal, J.; Abbasi, B.A.; Mahmood, T.; Kanwal, S.; Ali, B.; Shah, S.A.; Khalil, A.T. Plant-Derived Anticancer Agents: A Green Anticancer Approach. *Asian Pac. J. Trop. Biomed.* **2017**, *7*, 1129–1150. [CrossRef]
20. Kasture, V.; Gosavi, S.; Kolpe, J.B.; Deshapande, S.G. Phytochemical and Biological Evaluation of Fagonia Species: A Review. *WJPPS* **2014**, *3*, 1206–1217.
21. Irshad, S.; Anjum, I.; Mushtaq, M.N.; Ullah, K.; Barkat, K.; Hayat Malik, M.N.; Raza, M.A.; Zubair, M. Anti-Inflammatory, Analgesic and Anti-Pyretic Activity of Fagonia Bruguieri DC in Rats. *Pak. J. Pharm. Sci.* **2022**, *35*, 1209–1213.
22. Puri, D.; Bhandari, A.; Gaur, P. Screening of Anti-Histaminic Activity of Fagonia Schweinfurthii Hadidi in Guinea Pig Ileum and Formulate Anti-Histaminic Syrup. *Nat. Prod. J.* **2015**, *5*, 176–179. [CrossRef]
23. Rawal, A.K.; Muddeshwar, M.G.; Biswas, S.K. Rubia Cordifolia, Fagonia Cretica Linn and Tinospora Cordifolia Exert Neuroprotection by Modulating the Antioxidant System in Rat Hippocampal Slices Subjected to Oxygen Glucose Deprivation. *BMC Complement. Altern. Med.* **2004**, *4*, 11. [CrossRef] [PubMed]
24. Puri, D.; Bhandari, A. Fagonia: A Potential Medicinal Desert Plant. *J. NPA* **2015**, *27*, 28–33. [CrossRef]
25. Chopra, R.; Handa, K.; Kapur, L.; Chopra, I.C. *Indigenous Drugs of India*; Academic Publisher: New Delhi, India, 1994.
26. Parveen, R.F.I.M. Review on Medicinal and Bioactive Role of Genus Fagonia. *Fuuast J. Biol.* **2017**, *7*, 33–36.
27. Kakrani, H.N.; Saluja, A.K. Plants Used for Diuretic Activity in Traditional Medicine in Kutch Dis-trict, Gujarat. *J. Nat. Remedies* **2001**, *1*, 121–124. [CrossRef]
28. Said, H.M. *Medicinal Herbal: A Textbook for Medical Students and Doctors*; MAS Printers: Karachi, Pakistan, 1996; Volume 1, p. 29.
29. Wild Medicinal Plants of Madinat Al-Hikmah and Its Adjacent Areas. *Hamdard Med.* **1996**, *39*, 8–10.
30. Watt, G. Dictionary of the Economic Products of India Pages 1 to 178. In *A Dictionary of the Economic Products of India*; Cambridge University Press: Cambridge, UK, 2014; pp. 1–178, ISBN 9781107239142.
31. Hamid, A.; Majid, A.C.; Rehman, A. Isolation of Docosyl Docosanoate from Fagonia Cretica Linn. *Arab Gulf J. Sci. Res.* **1989**, *7*, 29–34.
32. Satpute, R.M.; Kashyap, R.S.; Deopujari, J.Y.; Purohit, H.J.; Taori, G.M.; Daginawala, H.F. Protection of PC12 Cells from Chemical Ischemia Induced Oxidative Stress by Fagonia Arabica. *Food Chem. Toxicol.* **2009**, *47*, 2689–2695. [CrossRef]
33. Ghasemi, M.; Turnbull, T.; Sebastian, S.; Kempson, I. The MTT Assay: Utility, Limitations, Pitfalls, and Interpretation in Bulk and Single-Cell Analysis. *Int. J. Mol. Sci.* **2021**, *22*, 12827. [CrossRef]
34. Liu, K.; Liu, P.-C.; Liu, R.; Wu, X. Dual AO/EB Staining to Detect Apoptosis in Osteosarcoma Cells Compared with Flow Cytometry. *Med. Sci. Monit. Basic Res.* **2015**, *21*, 15–20. [CrossRef]
35. Gul, R.; Jan, S.U.; Faridullah, S.; Sherani, S.; Jahan, N. Preliminary Phytochemical Screening, Quanti-tative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra Inter-media Indigenous to Balochistan. *Sci. World J.* **2017**, *2017*, 5873648. [CrossRef]
36. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. In *Oxidants and Antioxidants Part A*; Elsevier: Amsterdam, The Netherlands, 1999; pp. 152–178, ISBN 9780121822002.
37. Zhishen, J.; Mengcheng, T.; Jianming, W. The Determination of Flavonoid Contents in Mulberry and Their Scavenging Effects on Superoxide Radicals. *Food Chem.* **1999**, *64*, 555–559. [CrossRef]
38. Davies, N.W. Gas Chromatographic Retention Indices of Monoterpenes and Sesquiterpenes on Methyl Silicon and Carbowax 20M Phases. *J. Chromatogr. A* **1990**, *503*, 1–24. [CrossRef]
39. Irawan, C.; Rochaeni, H.; Sulistiawaty, L.; Roziafanto, A.N. Phyto-chemical Screening, LC-MS Studies and Antidiabetic Potential of Methanol Extracts of Seed Shells of *Archidendron bubalinum* (Jack) I.c. Nielson (Julang Jaling) from Lampung, Indonesia. *Pharmacogn. J.* **2018**, *10*, s77–s82. [CrossRef]
40. van Meerloo, J.; Kaspers, G.J.L.; Cloos, J. Cell Sensitivity Assays: The MTT Assay. *Methods Mol. Biol.* **2011**, *731*, 237–245. [CrossRef] [PubMed]
41. Singh, M.; Singh, R.K.; Singh, S.K.; Mahto, S.K.; Misra, N. In Vitro Biocompatibility Analysis of Functionalized Poly(Vinyl Chloride)/Layered Double Hydroxide Nanocomposites. *RSC Adv.* **2018**, *8*, 40611–40620. [CrossRef]
42. Sani, G.; Gualtieri, I.; Paolini, M.; Bonanni, L.; Spinazzola, E.; Maggiora, M.; Pinzone, V.; Brugnoli, R.; Angeletti, G.; Girardi, P.; et al. Drug Treatment of Trichotillomania (Hair-Pulling Disorder), Excoriation (Skin-Picking) Disorder, and Nail-Biting (Onychophagia). *Curr. Neuropharmacol.* **2019**, *17*, 775–786. [CrossRef]

43. Kumar, N.R.; Reddy, J.S.; Gopikrishna, G.; Solomon, K.A. GC-MS determination of bioactive constituents of Cycas beddomei cones. *Int. J. Pharm. Bio. Sci.* **2012**, *3*, 344–350.
44. Venkata, R.B.; Samuel, L.A.; Pardha, S.M.; Narashimha, R.B.; Naga, V.K.; Sudhakar, M.; Radhakrishnan, T.M. Antibacterial, Antioxidant Activity and GC-MS Analysis of Eupatorium Odoratum. *Asian J. Pharm. Clin. Res.* **2012**, *5*, 99–106.
45. Lewis, R.A. *Hawley's Condensed Chemical Dictionary*, 16th ed.; Larranaga, M.D., Lewis, R.J., Eds.; Wiley: New York, NY, USA, 2020; ISBN 9781119371755.
46. El-Demerdash, E. Anti-Inflammatory and Antifibrotic Effects of Methyl Palmitate. *Toxicol. Appl. Pharmacol.* **2011**, *254*, 238–244. [CrossRef]
47. Sutanto, H.; Susanto, B.H.; Nasikin, M. Solubility and Antioxidant Potential of a Pyrogallol Derivative for Biodiesel Additive. *Molecules* **2019**, *24*, 2439. [CrossRef]
48. Sehat, N.; Yurawecz, M.P.; Roach, J.A.G.; Mossoba, M.M.; Eulitz, K.; Mazzola, E.P.; Ku, Y. Autoxidation of the Furan Fatty Acid Ester, Methyl 9,12-Epoxyoctadeca-9,11-Dienoate. *J. Am. Oil Chem. Soc.* **1998**, *75*, 1313–1319. [CrossRef]
49. Preedy, V.R.; Watson, R.R. *Olives and Olive Oil in Health and Disease Prevention*; Academic Press: San Diego, CA, USA, 2020; ISBN 9780128199893.
50. Dilika, F.; Bremner, P.D.; Meyer, J.J. Antibacterial Activity of Linoleic and Oleic Acids Isolated from Helichrysum Pedunculatum: A Plant Used during Circumcision Rites. *Fitoterapia* **2000**, *71*, 450–452. [CrossRef] [PubMed]
51. Moorthy, B.; Chu, C.; Carlin, D.J. Polycyclic Aromatic Hydrocarbons: From Metabolism to Lung Cancer. *Toxicol. Sci.* **2015**, *145*, 5–15. [CrossRef]
52. UCSD/CCMS—Spectrum Library. Available online: http://gnps.ucsd.edu/ProteoSAFe/gnpslibraryspectrum.jsp?SpectrumID=CCMSLIB00000078682 (accessed on 13 January 2023).
53. Falzone, L.; Salomone, S.; Libra, M. Evolution of Cancer Pharmacological Treatments at the Turn of the Third Millennium. *Front. Pharmacol.* **2018**, *9*, 1300. [CrossRef]
54. El-Negoumy, S.I.; Al-Wakeel, S.A.M.; El-Hadidi, M.N.; Saleh, N.A.M. The Flavonoids of the Fagonia Arabica-Complex (Zygophyllaceae). *Phytochemistry* **1986**, *25*, 2423–2424. [CrossRef]
55. El-Wakil, E.A. Phytochemical and Molluscicidal Investigations of Fagonia Arabica. *Z. Naturforsch. C* **2007**, *62*, 661–667. [CrossRef]
56. Tawaha, K.; Alali, F.; Gharaibeh, M.; Mohammad, M.; Elelimat, T. Antioxidant Activity and Total Phenolic Content of Selected Jordanian Plant Species. *Food Chem.* **2007**, *104*, 1372–1378. [CrossRef]
57. Pandey, A.K.; Kumar, S.; Pandey, A.K.; Reis, F. Editorial: Combating Redox Imbalance-Associated Complications with Natural Products. *Front. Pharmacol.* **2021**, *12*, 802750. [CrossRef]
58. George, S.; Abrahamse, H. Redox Potential of Antioxidants in Cancer Progression and Prevention. *Antioxidants* **2020**, *9*, 1156. [CrossRef]
59. Iftikhar, N.; Chatha, S.A.S.; Ahmad, T.; Ali, Q.; Hussain, A.I.; Rathore, H.A. Fagonia Arabica L.: A Review of Its Phytochemistry, Pharmacology and Traditional Uses. *Comb. Chem. High Throughput Screen.* **2022**, *25*, 1187–1199. [CrossRef]
60. Patel, D.K.; Singh, R.K.; Singh, S.K.; Aswal, V.K.; Rana, D.; Ray, B.; Maiti, P. Graphene as a Chain Extender of Polyurethanes for Biomedical Applications. *RSC Adv.* **2016**, *6*, 58628–58640. [CrossRef]
61. Rooprai, H.K.; Lawrence, P.; Keshavarz, S.; Yashod, P.; Gullan, R.W.; Selway, R.P.; Davies, D. DRAQ7 as an Alternative to MTT Assay for Measuring Viability of Glioma Cells Treated with Polyphenols. *Anticancer Res.* **2020**, *40*, 5427–5436. [CrossRef] [PubMed]