In Vitro Antiproliferative Effect of *Ficus exasperata* (Vahl) on Ovarian Cancer Cells –a Preliminary Investigation

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ABSTRACT

Ovarian cancer is one of the most common gynaecological cancers today. Due to the high relapse rates (75%) in advanced ovarian cancer cases and the increasing resistance to currently available treatments, research into the development of new therapies is therefore required. This study therefore aims to evaluate the anticancer effects of *Ficus exasperata* extracts and fractions on ovarian cancer cells and to identify some of the active components.

The antiproliferative activity of the crude extracts (1 mg/mL) was assessed using the MTT assay on A2780 (ovarian cancer) cell line. Fractionation of the chemical constituents by flash chromatographic technique was guided by the antiproliferative activity. Identification was further achieved using high resolution mass spectrometry and nuclear magnetic resonance spectroscopy. Observation of the dead cells was carried out via microscopy.

All crude extracts tested exhibited antiproliferative activity except for the first phase of the methanol extract (**MFF1**) which interestingly showed proliferative effects. Five fatty acids were identified from the active fractions (**FB1-10** and **FB1-12**). **FB1-12** exhibited an IC_{50} value of 15.20 μg/mL. The least potent fraction (FB1-4+5) had an IC_{50} value of 34.51 μg/mL. Both **H1-HEX** and **H1-MET** displayed inhibitory effects (97.2% and 97.9% respectively) compared to control.

It can therefore be concluded that *Ficus exasperata* extracts exhibit significant antiproliferative and cytotoxic effects on ovarian cancer cells.

Keywords: *Ficus exasperata*; ovarian cancer; MTT assay; A2780 cells; Flash chromatography; Fatty acids; Hexane; Ethylacetate; Methanol; NMR

1. Introduction

Ovarian cancer (OC) is an overarching term for malignancies originating from different cell types within the ovaries. It has become one of the great challenges faced by clinicians in gynaecologic oncology [1]. It currently stands as the eighth most common cancer in women and the seventh most common cause of cancer death [2]. Many theories attempt to explain the pathological processes involved in OC; each has its' limitations and no single theory can completely explain all aspects of the disease process. Persistent ovulation is a possible cause due to repeated trauma, repair and remodelling of the tissues within the ovaries [3]. This may increase the risk of mutational changes upon repair. Another theory proposes opposing effects of androgens and progestogens; androgens resulting in increased tumour formation and progestogens exerting a protective effect [3]. Genetic mutations have also been observed to exist in a small proportion of individuals who develop OC [1]. A lack of disease-specific symptoms can make OC diagnosis difficult (symptoms may be confused with conditions such as endometriosis and menstruation) and so a large proportion of diagnoses are made at advanced stages (around 62%) [4] complicating therapy. It has also been estimated that only 15% of cancers remain localised to the ovary at the time of diagnosis; this further complicates treatment [4].

Despite improvements in OC treatment over the past decades, it is obvious that further research is needed in order to improve mortality and survival rates. This is evidenced in the fact that 75% of patients with advanced OC, relapse after initial treatment [5]. Current treatments may well induce remission; however, relapse rates indicate that these treatments are not as effective as intended. Research should focus on both novel therapies and optimisation of current therapies [5]. Increasing need for new drug therapies and treatments for OC has resulted in increases in natural products research. For instance, the anticancer effects of green

tea and its' components have indicated promising results *in vitro* [6]. Traditional Chinese medicine also holds good promise for new anticancer drug development leads [7].

The *Ficus* species are an emerging group of plants with promising potent anticancer constituents. *F. carica, F. religiosa and F. septica* have been widely studied [8–10]. Positive cytotoxicity results with these *Ficus* species suggest that *Ficus exasperata* (*FE*) could be another potential area for the development of novel anticancer agents. Previous research on *FE* has been centred around effects on gynaecological structures and toxicity [11,12]. Today, there are currently unmet clinical needs with regards to the treatment of OC. This study is therefore aimed at the identification of potential anticancer activities of a variety of extracts from *FE* in an OC cell line (A2780) and the identification of the compounds from these active extracts. To our knowledge, this is the first report on the antiproliferative effect of *FE* on ovarian cancer cell lines.

2. Materials and methods

2.1 *Plant Material Extraction*

The leaves, figs and stem bark of *FE* were collected in September, 2010 from Benin City, Nigeria. The plant was previously authenticated by Mr. Felix Usang of the Forest Research Institute of Nigeria (FRIN), Ibadan, with a voucher number of F.H.I. 107312. Voucher specimen were prepared and deposited at FRIN and at the Department of Pharmacognosy, University of Benin, Nigeria. The fresh plant materials were cleaned, dried and powdered. The figs (79 g), stem bark (450 g) and leaves (500 g) were macerated and constantly stirred in acetone, ethyl acetate (EtOAc), methanol and hexane, each for 72 h. Following maceration, each extract was filtered and concentrated under a vacuum. Extracts were weighed and stored at -20°C until required. Extraction of figs produced three extracts: the acetone fig extract

(**AFF1**) (10.5 mg), the ethyl acetate *Ficus* fig extract (**EFF1**) (20.5 mg) and the methanol *Ficus* fig extract (**MFF1**) (23.1 mg); Extraction of the stem bark produced three major extracts: the *Ficus exasperata* hexane bark extract (**FEBH**) (98 mg), the *Ficus exasperata* ethyl acetate bark extract (**FEBE**) (86 mg) and the *Ficus exasperata* methanol bark extract (**FEBM**) (70 mg). Extraction of the leaves with hexane (using a Soxhlet extractor set at 40°C) produced the hexane extract (**H1**) (19.23 g). All solvents used were obtained from Fisher Scientific, UK (HPLC grade unless otherwise stated).

2.2 In Vitro Anticancer Assay of Crude Extracts

2.2.1 Cell Line Culture

A2780 human ovarian carcinoma cell lines (originally purchased from Sigma Aldrich, UK) were cultured in 75 cm^2 Corning flasks (Corning Inc, NY) prior to assay. Cells were cultured in media (RPMI 1604 supplemented with 10% Foetal Bovine Serum (PAA Laboratories, Austria) and 1% concentrations of Penicillin, Streptomycin, Glutamine and Sodium Pyruvate (all Sigma Aldrich, UK)). Incubation conditions of 37 °C and 5% CO_2 were maintained throughout. Media was removed and cells washed with 5 mL of Hanks Balanced Salt Solution (Life Technologies, UK) twice. Two minutes incubation with Trypsin (5 mL) was allowed for removal of adherent cells from the flask walls. Centrifugation at 1000 rpm for 2 min produced a pellet of cells and a supernatant (removed). After addition of 10 mL of media, cells were counted using a counting slide and microscope (Nikon TMS microscope fitted with Moticam (800 x 600 pixels)) to determine the cell solution volume to be added to each well to yield 20,000 cells/well. Cell distribution was examined under the microscope and then allowed to attach and recover within a 24 h incubation period.

2.2.2 Sample Preparation and MTT Antiproliferative Assay

Stock solutions of 20 mg/ml were prepared using approximately 100 mg of extract in an appropriate volume of dimethylsulphoxide (DMSO). The extract stock solutions were diluted to produce a final concentration of 1 mg/ml which were added to the respective wells of the 96-well plate. Quadruplicates were used in all assays. Control, blank, and vehicle controls (100% DMSO and 1% DMSO) were also plated (minimum of 4 wells each). After inoculation, the plate was incubated for 24 h. The yellow tetrazolium salt 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) antiproliferative assay was performed after incubation. The principle applied involves the ability of metabolically active cells being able to convert the dye to water-insoluble dark blue formazan and in the process reducing cleavage of the tetrazolium ring [13]. MTT was dissolved in Phosphate Buffer Solution to produce a 5 mg/mL solution; it was then filtered using Millex Syringe Driven Filter Unit (0.22 μm) before being combined with media to produce 10 mL MTT-media solution (20% MTT : 80% media). Media and extracts were aspirated from the 96 well plate prior to addition of 100 μL/well of MTT-media solution. Cells were checked under the microscope and then incubated for 4 h. After incubation, the MTT-media solution was removed and 150 μL/well DMSO was added to solubilise the formazan products formed. After 5 min incubation with 100% DMSO, the optical density of the plate was read using an ELISA reader and Softmax Pro software. The optimum wavelength was read with respect to the reference wavelengths (550 nm and 650 nm respectively). Results were converted to % cell inhibition due to extract presence compared to control (% inhibition = ((1-(Average AU_{sample}/Average $AU_{control}$)) x 100).

2.3 Chromatographic Analysis of Active Extracts

The active extracts selected from the cytotoxicity analysis which included all extracts except **MFF1**, were analysed on Thin Layer Chromatography (TLC) plates (Silica Gel 60 F_{254}, Merck, Germany). A 5 cm run was used with a hexane:EtOAc solvent system (plate one 70:30 and plate two 90:10). Samples were dissolved in a small amount of EtOAc. Bergapten, (previously identified from the stem EtOAc extract) was also spotted for comparative purposes. Plates were subsequently visualised under both short and long wave UV light and retention factor (Rf) values calculated (Rf = spot height/solvent front height).

After fractionation (described below), **H1**, **H1-HEX** and **H1-MET** were analysed on TLC plates produced as previously described. Solvent systems were as follows: plate one EtOAc:methanol (90:10), plate two hexane:EtOAc (90:10) and plate three hexane:methanol (95:5). Different solvent systems were utilised in order to allow for future use of the system which yielded the best Rf value. Plates were visualised as previously described. **FB1** fractions were analysed on TLC plates using the hexane:EtOAc (90:10) solvent system.

2.4 Purification and Fractionation of Active Extracts

H1, and **FEBE** were found to be most active from bioassay results. **FEBE** was further shortened to **FB1**. Further fractionation and separation procedures were then performed.

Liquid/liquid partitioning of **H1** was carried out using hexane and aqueous methanol ((AM) 10% water and 90% methanol) in a separatory funnel. The AM phase was collected (after shaking and settling) into a conical flask and then replaced. This was repeated three times and both phases were collected and filtered. After filtration, the AM and hexane phases were concentrated using a Rotavapor R3, set at a temperature of 40°C. These were labelled **H1-MET** and **H1-HEX** and assayed, as previously described, at a concentration of 100 µg/ml.

FB1 was prepared for dry loading flash chromatography by combining 1 g **FB1** dissolved in 1.8 ml EtOAc with 1 g Celite ® (Sigma Aldrich, UK). A step-wise gradient system utilizing two solvent-system combinations (hexane and EtOAc) in each run was performed for **FB1** elution using a flash chromatogram. The mixture was dried overnight and loaded into a 12 g column and run using a flash chromatogram set at flow rate of 10 mL/min, run length 90 min, ELSD threshold 5 mV, UV threshold 0.05AU, UV_1 245 nm, UV_2 280 nm and per-vial volume 5 mL. The step gradient elution was as follows: step 1: hexane (A)-EtOAc (B) (100% A -0% B changing to 90% A- 10% B in 45 min). Step 2: (90% A -5% B changing to 70% A-30% B in 45 min). Fractions were collected in volumes of 50 mL. About 500 fractions were collected and subsequently pooled by TLC (a 4 cm run was used with 0.5 cm spot spacing and a 90:10 hexane:EtOAc solvent system). Every second fraction was tested. Fractions were pooled based on similarities under UV light. A total of 17 fractions were obtained (**FB1-1** to **FB1-18**, with **FB1-4** and **FB1-5** combined due to similarities in the NMR spectra). All fractions were tested using the MTT antiproliferative assay, as described above, using a 1 mg/mL stock solution. FBC range 1-100 μg/mL was used to produce concentration-inhibition profiles and compute IC_{50} and log IC_{50} values.

2.5 ^{1}H-NMR and MS Analysis of Extracts and Fractions

Extracts were prepared for Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS) analysis. Approximately 5-10 mg of each extract was dissolved in 700 μl of deuterated chloroform ($CDCl_3$) (Sigma Aldrich, UK). **H1-HEX**, **H1-MET** and **FB1** fractions were constituted in $CDCl_3$ (600 μl) for ^{1}H-NMR analysis. An AS-400 JEOL NMR instrument operating on an eclipse-400 spectrometer at 23˚C equipped with a 40TH5AT/FG broadband high sensitivity Pulse Field Gradient 'AutotuneTM' 5 mm probe was used to

analyse samples. Settings were as follows: 400 MHz at 23˚C on an eclipse-400 spectrometer. 1D-NMR spectra were obtained using the following parameters; 16-32k data points, spectral width of 7199 and pulse width of 5.4750 Hz respectively, an acquisition time of 1.14 min and a relaxation delay of about 2.0 s.

Prior to preparation for MS analysis, the extracts and fractions were dried under nitrogen, 50 µl dichloromethane and 450 µl methanol were then added to the MS vials (only methanol (500 µl) was added to **MFF1**, and **FEME**). A 500 µl blank was prepared for each solvent mixture. Mobile phases were prepared (1l of 0.1% formic acid in distilled water and 0.8L of 0.1% formic acid in acetonitrile) for use in MS analysis. A hyphenated Exactive Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific Inc., UK) set at 22˚C was used to perform HPLC-HRMS (both positive and negative ion modes) on extracts and fractions. A silica type 75.0 x 3.0 mm C18 ACE HPLC column (Hichrom Limited, UK) was employed. Particle size of the silica was 5 µm, pore size 100Å with a pore volume of 1.0 ml/g and a surface area of 300 m^2/g which was maintained at a temperature of 22°C and an approximate pressure of 37 bar. The column was eluted with a linear gradient of 90% A (0.1% v/v formic acid in water) and 10% B (0.1% v/v formic acid in acetonitrile) mobile phases with a flow rate of 0.3 ml/min over 0-35 min then to 100% B for a further 5-min isocratic elution, and a return to starting conditions at 40 min for re-equilibration for the last 5 min, using up a total of 45 min for the run. A 10 µl injection volume was used and the mass spectrometer was operated with UV detection at 254 nm. Approximately 1 mg of the extracts was dissolved in 1 ml HPLC grade methanol and solutions containing insoluble materials were filtered to avert blockade of the sample introduction line. The samples were transferred to 1.5 ml glass HPLC auto sampler vials from which the Agilent 1100 auto sampler withdrew samples that were set at 200 µl/min ejection speeds, one wash cycle and 20 wash strokes.

MS data were processed and analysed using MZMine and Dictionary of Natural Products while the ^{1}H-NMR spectra were processed and analysed using MNova v.8.0 software.

2.6 Statistical Analysis

All data were expressed as mean ± standard error of mean (SEM). Concentration-response curves were produced using GraphPad Prism v. 6.0 (SanDiego, CA) for each extract. IC_{50} values were calculated using the equation: $\text{Response} = (\text{Bottom} + (R_{max} - \text{Bottom}))/(1 + 10^{(\text{Log } IC50 - \text{Log concentration})})$.

3. Results

3.1 Cell Inhibition Profile of Extracts and FEBH

It was observed that all extracts tested exhibited greater than 80% cell growth inhibition, except for **MFF1** which displayed cell growth enhancement (Fig. 1). Results of this cell growth inhibition assay assisted in selection of extracts for further study.
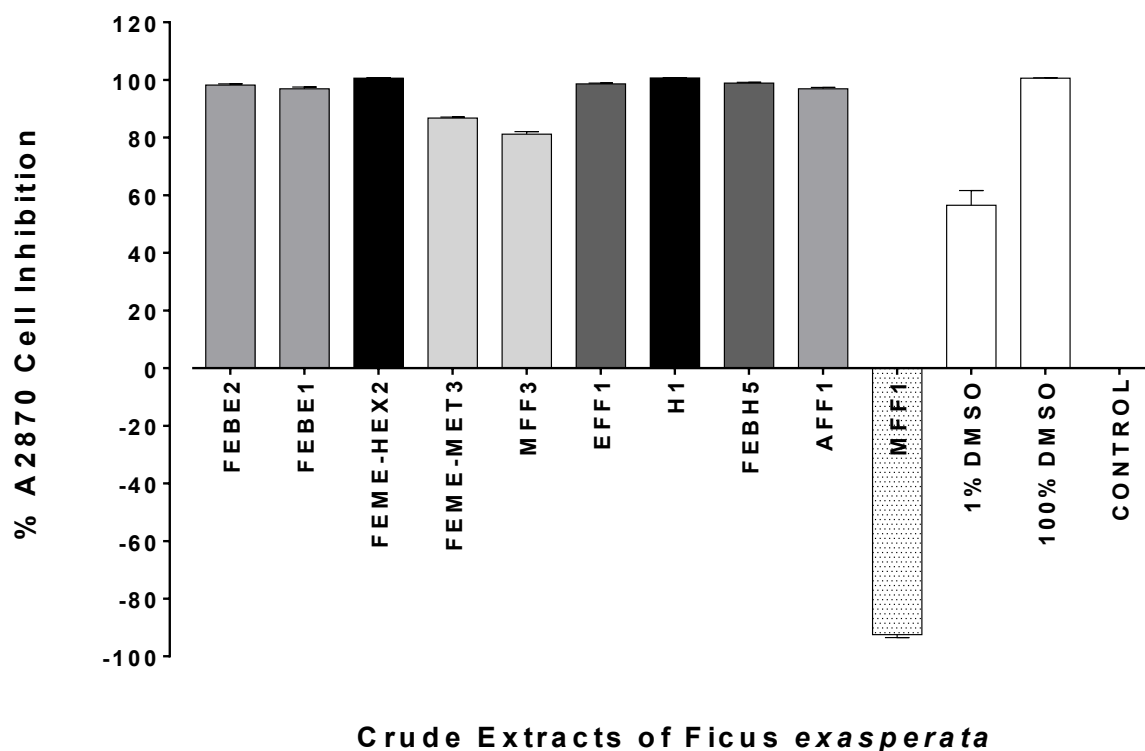
Figure 1: Mean % A2780 cell inhibition after incubation with all crude Ficus *exasperata* extracts (figs, stem bark and leaves) at a concentration of 1 mg/mL for 24 h. Compared to control, mean % inhibition ranged from -92.5% to 100.7%. Experiments were completed in quadruplicate and results displayed as mean ± standard error mean (some error bars too small to be seen). (extracts n=4, controls n=8).

3.2 Cell Inhibition Profile of FEBE Fractions

It was observed that all fractions exhibited good inhibitory activity at higher concentrations of 75 and 100 μg/ml (Fig. 3A-D). Curve-fitting allowed for IC_{50} value calculation for each of the fractions and enabled for comparison of each fraction's activity.

Log IC_{50} values ranged between 1.182 μg/mL and 1.538 μg/mL (Fig. 4). There were no statistically significant differences between the log IC_{50} values of all **FB1** fractions; an indication that each fraction possesses equipotent effects on A2780 cell growth.
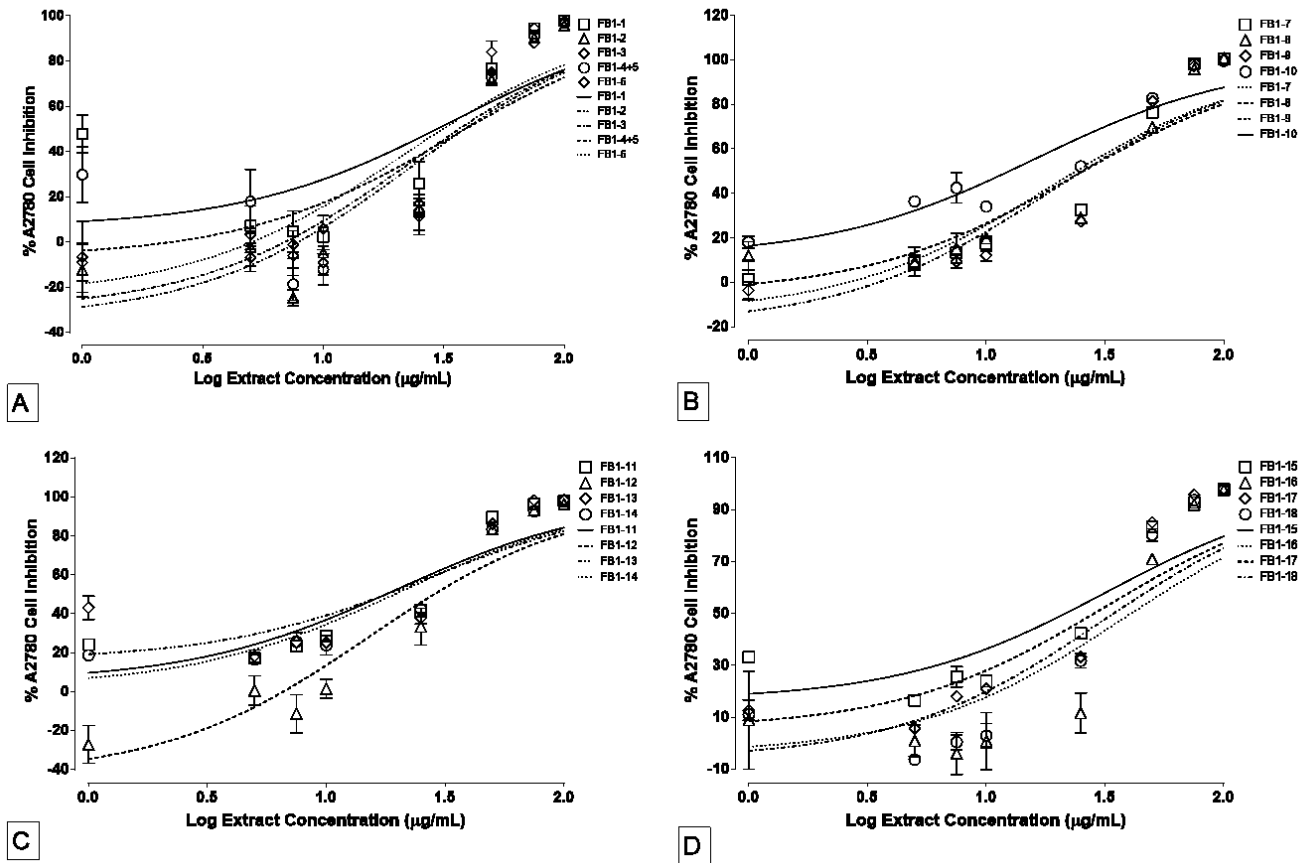
Figure 3(A-D): Concentration-Response curves for **FB1** fractions. Inoculation (24 h) with extract concentrations ranging from 1-100 µg/mL resulted in % A2780 cell inhibition values ranging -27.1% to 100.9% (compared to control). EC_{50} values between 15.2 and 34.51 µg/ml. Experiments were completed in quadruplicate and results displayed as mean ± standard error mean (n=32).
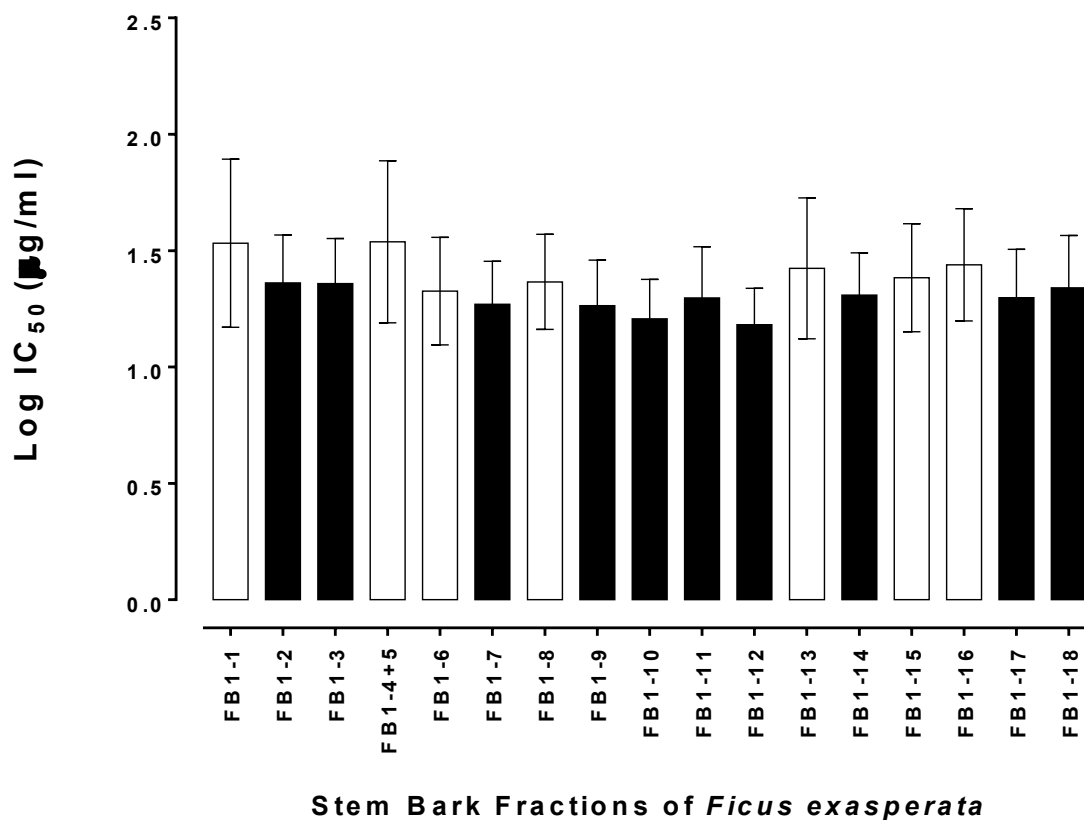
Figure 4: Comparison of log IC_{50} values for each of the **FEBE1** fractions. Experiments completed in quadruplicate with results displayed as mean ± standard error mean (n = 32). One-way ANOVA with Tukey's multiple comparison test yielded no significant differences between the log IC_{50} values of any of the fractions (p = 1.00, significance at p<0.05).

3.3 Cell Inhibition Profile of Partitioned H1

Inhibitory effects of **H1-HEX** and **H1-MET** were also analysed at a single concentration of 100 μg/ml. It was observed during the screening stage that both **H1** fractions exerted significant inhibitory effects on A2780 cells (Fig. 5). The results were similar to those of the **FB1** fractions at 100 μg/mL (Fig. 3).
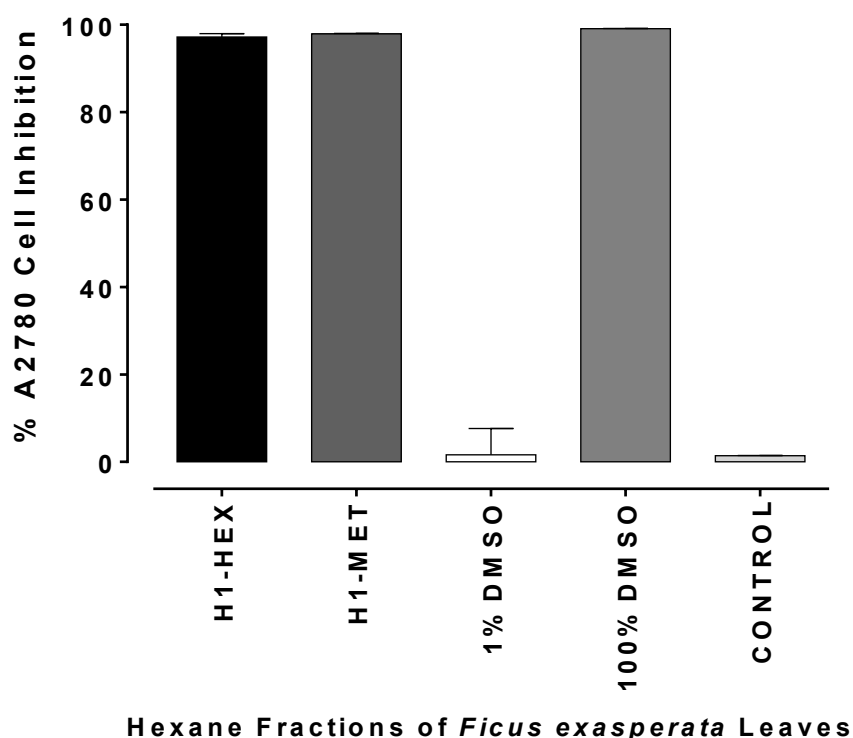

Figure 5: Mean % A2780 cell inhibition exhibited after incubation with 100 mg/mL **H1-HEX** and **H1-MET** extracts for 24 h. Mean % cell inhibition values of 97.2% and 97.9% were obtained for **H1-HEX** and **H1-MET** respectively, compared to control. Inhibition values attributed to 1% DMSO and 100% DMSO were 1.6 % and 99.1% respectively. Experiments were completed in octuplicate and results displayed as mean ± standard error mean (some error bars too small to be seen). (n=8)

3.4 Cell Morphology Examination

Microscopic examination of cells was also carried before and after inoculation of the A2780 cells with *FE* fractions. Visual comparisons allowed for crude detection of morphological changes as well as changes in cell density. On the most part, visual findings were congruent with bio-assay data. Reductions in cell numbers, cell lysis and clumping of cells after addition of the active samples were observed (Fig. 6).
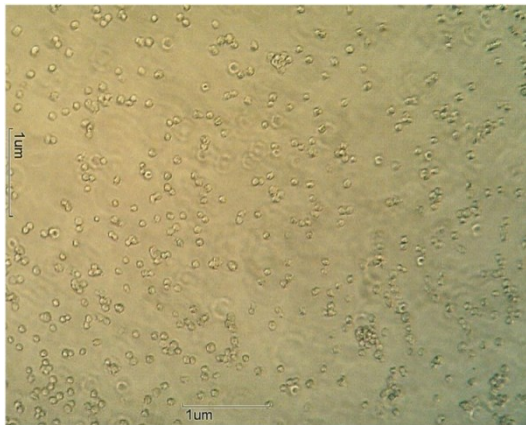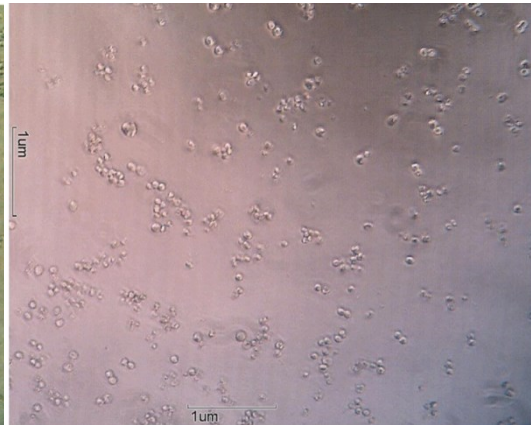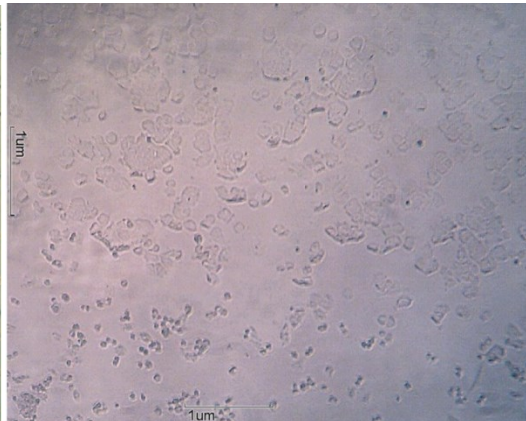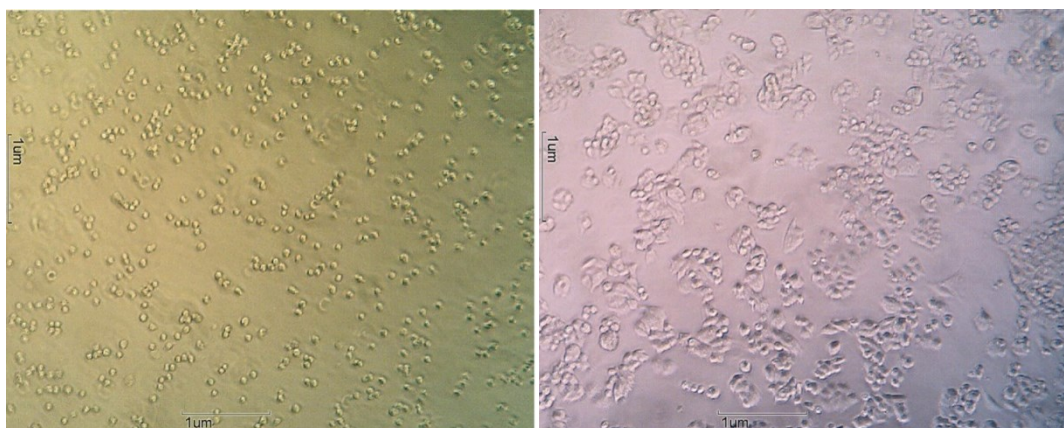

A1   A2
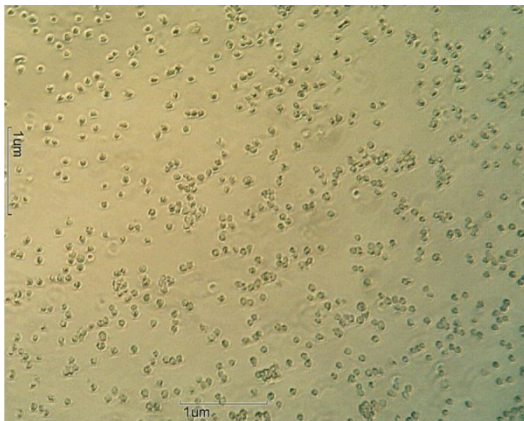
B1   B2

C1   C2

Figure 6: Images of A2780 cells (passage #40 and x10 magnification) using Moticam 800 x 600 pixels with Nikon TMS microscope, before (left) and after inoculation and incubation (right). A1 and A2 represent cells before and after inoculation with FB1-16, B1 and B2 before and after inoculation with 100% DMSO and C1 and C2 are controls (before and after 24 h incubation). Scale bars denote 1 μm.

3.5 Identification of Chemical Constituents

Preliminary identification of the active **FB1** fractions was performed utilizing both MS and NMR analyses. Focusing on the two most active fraction, fraction **FB1-12** was the most potent cytotoxic fraction among the 18 **FB1** fractions investigated with the Log IC_{50} value at 1.18 ± 0.15. Structural characterization of this fraction revealed the presence of two major compounds, bergapten and a fatty acid. Bergapten EI-MS: m/z 217.0499 [M^+, $C_{12}H_8O_3$] (Fig. 7). ^{1}H-NMR (CDCl$_3$, 400 MHz): δ8.15 (H-4, d, J= 9.8 Hz), δ7.57 (H-7, d, J= 2.5Hz), δ7.12 (H-9, s), δ7.01 (H-6, d, J= 2.5 Hz), δ6.25 (H-3, d, J= 9.8 Hz), δ4.25 (singlet, 5-OCH$_3$). The second compound was identified as oxo (5:1/5:0/8:0)] (1S, 2S)-3-oxo-2-(2'Z-pentenyl)-cyclopentaneoctanoic acid EI-MS: m/z 295.2276 [M+1 $C_{18}H_{30}O_3$] (Fig. 7). ^{1}H-NMR (CDCl$_3$, 400 MHz): δ4.12 (H-, m); δ2.33 (H-, t, J=7.55 Hz), δ2.08 (H-, d, J= 10.52), δ1.32, s), δ0.87, t, J= 5.90). A minor

compound was additionally identified via MS and Mzmine as 3beta-Hydroxy-4beta-methyl-5alpha-cholest-7-ene-4alpha-carboxylate EI-MS: m/z 445.3678 [M^+1 $C_{29}H_{48}O_3$] (Fig. 7).

The next potent fraction was **FB1-10** with Log IC_{50} values at 1.207 ± 0.17 (Fig. 4). Structural characterization of **FB1-10** revealed the presence of two major compounds, Jasmonic acid and [18:4]6z,9z,12z,15z octadecatetraenoic acid. The EI-MS of Jasmonic acid showed an m/z at 211.133 [M^+ $C_{12}H_{18}O_3$] (Fig. 8). ^{1}H-NMR (CDCl$_3$, 400 MHz): δ5.48- 5.24 (m, 2H, H-10 and H-9), δ 2.80 (dd, J = 8.08 and 13.77 Hz, 1H, H-4), δ 2.42-2.29 (m, 5H, H-8, H-6 and H-5α), δ2.05 (dd, J = 10.20 and 9.0 Hz, 2H, H-11), δ1.84 (m, 1H, H-2), δ1.65 (ddd, J = 7.32; 7.91 and 14.66 Hz, 1H, H-5β), δ0.90 (m, H-12). The EI-MS of octadecatetraenoic acid showed an m/z at 293.2118 [M^+ $C_{12}H_{28}O_3$] (Fig. 8) and the ^{1}H-NMR spectra corresponds to identification in the literature [14].
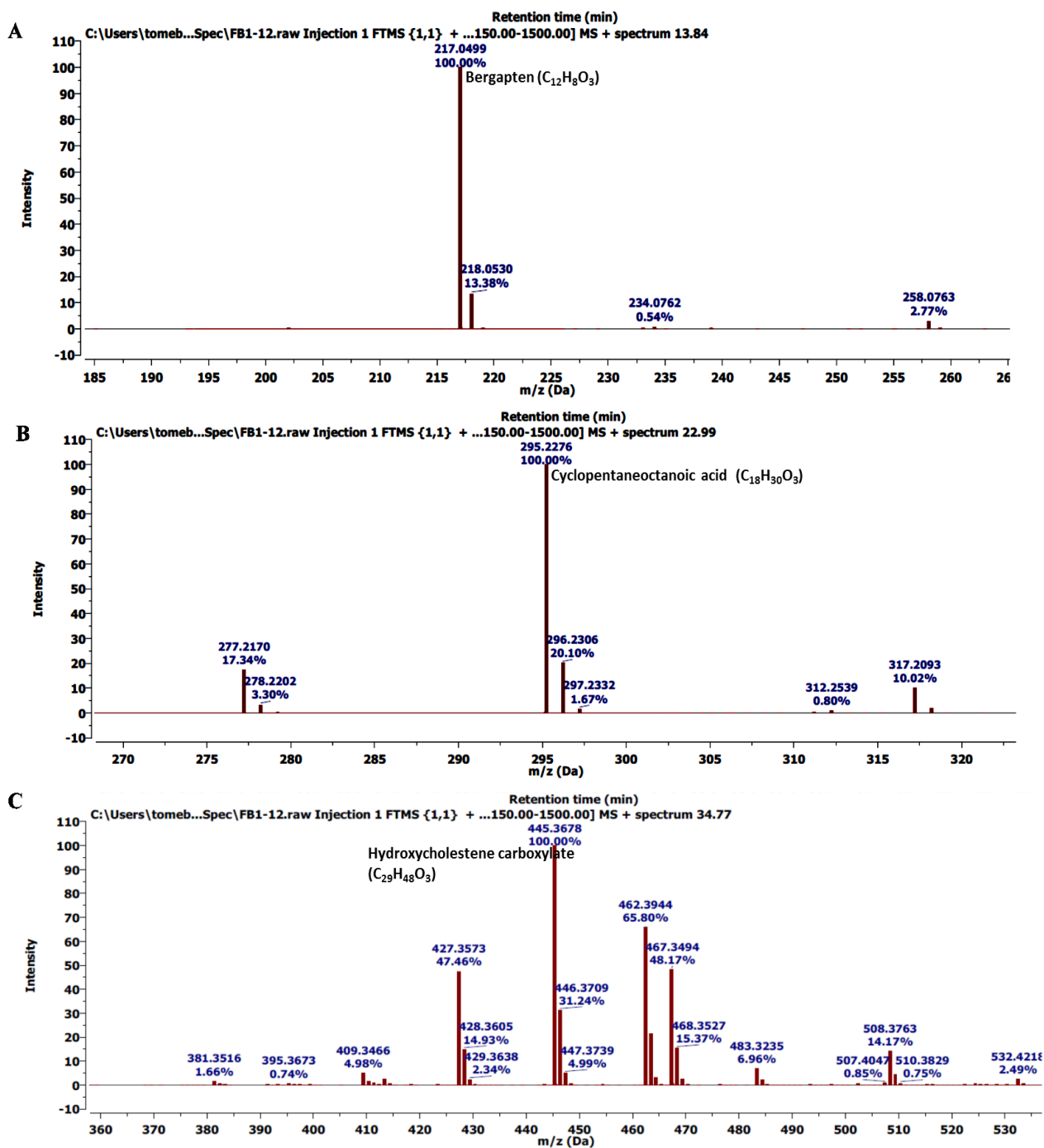
Figure 7. Positive HRESI-MS chromatograms showing the intensity (y-axis) and the m/z (x-axis) of **FB1-12**. (A) Bergapten, (B) Cyclopentaneoctanoic acid, (C) Hydroxycholestaene carboxylate.
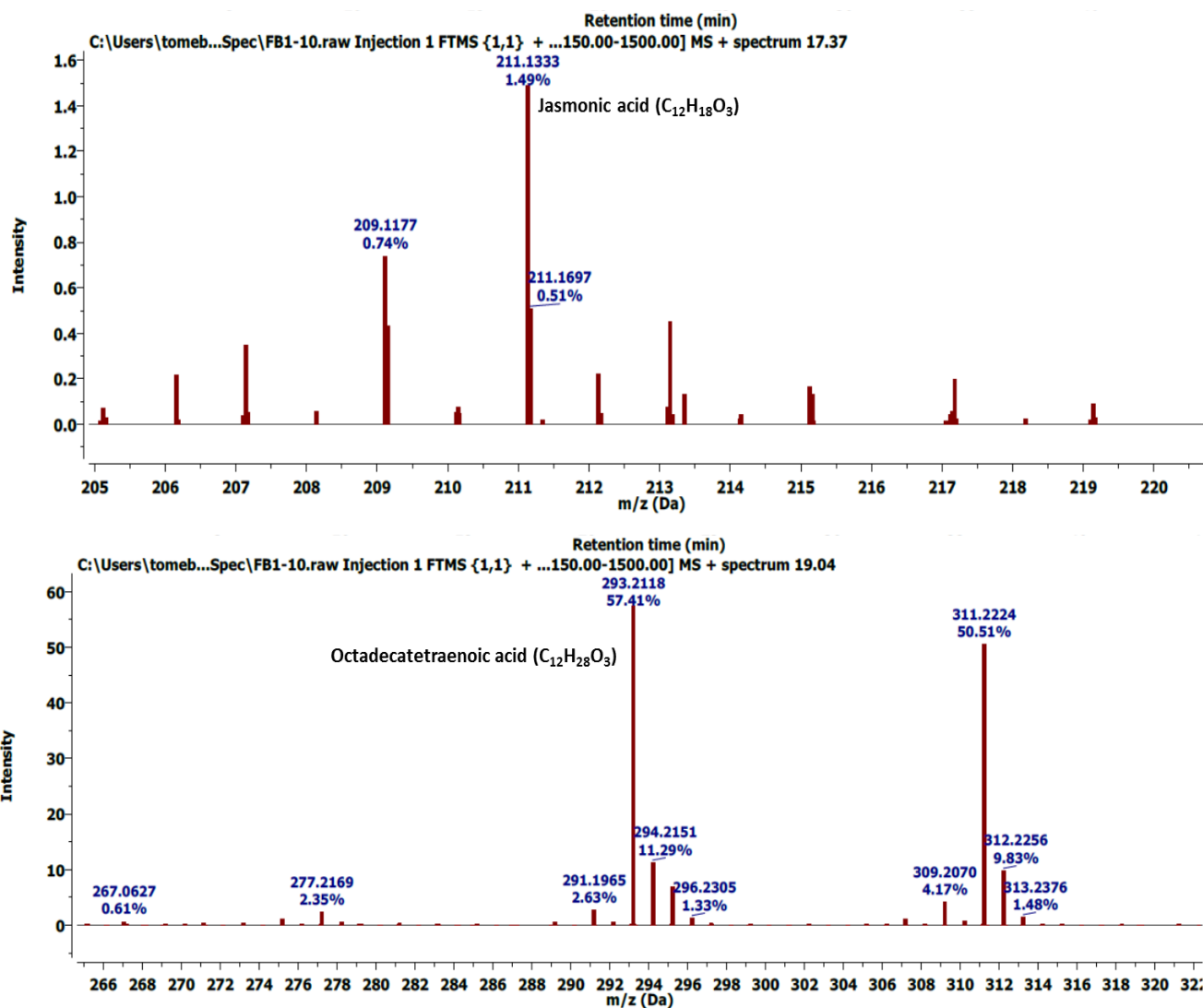
Figure 8. Positive HRESI-MS chromatograms showing the intensity (y-axis) and the m/z (x-axis) of FB1-10. (A) Jasmonic acid, (B) Octadecatetraenoic acid.

4. Discussion

This study to our knowledge reports the first *in vitro* anticancer study of *FE* extracts on ovarian cancer cell lines. FE is the least studied *Ficus* species [15] but yet appears to hold potential in medicinal effects. Examination of the NMR spectra and MS data, collected from **FB1** fractions, revealed the presence of fatty acids (FAs) which appear to play a role in the antiproloferative effects observed in this study. The impact of FAs intake has been evaluated in terms of cancer

development risk and cancer treatment. Reductions in cancer development risk have been observed upon long-term intake of long-chain polyunsaturated FAs (LCPUFAs) [16]. Addition of LCPUFAs to currently used chemotherapeutic agents resulted in improved clinical effectiveness and reduced non-target tissue toxicity [16]. It has been proposed that anticancer activity of LCPUFAs is mediated by an anti-inflammatory effect. LCPUFAs from marine sources has been associated with potential risks and unpleasant side-effects [16]. Therefore, *FE* may enable production of FAs displaying anticancer effects from a non-marine source with potentially less or no associated risks. One study observed no benefit after administration of omega-3 FAs in terms of OC risk reduction [17], whilst some FAs may possibly be associated with the development of resistance to cancer chemotherapy [16]. The available evidence is inconclusive; however, it appears to favour the possibility of some FAs possessing anticancer activity. In addition to the fatty acids discovered in this study was jasmonic acid which belongs to the family of plant stress hormones that are able to activate cellular responses (cell death inclusive) in response to stress conditions in plants, and can also subdue the proliferation process of cells or cause apoptosis in different types of cancer cells without affecting the normal cells [18]. Jasmonates occur universally in plants but not in animals [19, 20]. Jasmonates such as 12-oxo phytodienoic acid (OPDA), jasmonic acid (JA), methyl jasmonate (MeJA), jasmonoyl isoleucine (JA-Ile), and 12-hydroxy-jasmonic acid (12-OH-JA) are ubiquitously occurring signaling compounds formed in plants in response to abiotic and biotic stresses [21]. Linolenic acid is a precursor of Jasmonates and they are synthesized through an octadecanoid pathway. They can therefore be regarded as plant-derived oxylipins similar to mammalian oxylipins such as prostaglandins and leukotrienes with which they share structural similarities [22]. Prostaglandins and leukotrienes also play a role in stress conditions. Jasmonic acid and derivatives play a role in the regulation of plant immunity, germination, and development [21] as well as in plant defense against oxidative stress which may be due to injury or ozone

exposure [23]. Recently the anti-cancer properties of jasmonates have been intensively studied [18, 26–29]. plants with high content of jasmonic acid such as *Viscum album* are currently of pharmaceutical interest [30].

Currently used OC chemotherapeutic agents, such as cisplatin, can be compared, in terms of inhibitory effects, to the **FB1** fractions. Work by Kieger *et al.* (2010) observed an IC_{50} value of 16.41 μM (4.94 μg/mL) for cisplatin using A2780 cells (under the same conditions used in this research) [31]. This current study observed that **FB1-12** was the most potent fraction (IC_{50} value of 15.20 μg/ml). It is very promising to note that an unrefined *FE* fraction has approximately one third of the inhibitory activity of cisplatin. Increasing levels of resistance to commonly used anticancer agents make this research valuable. It has been observed that treatment of cisplatin-resistant A2780 cells with cisplatin resulted in an IC_{50} value of 74.97 μM (22.6 μg/ml) [31] displaying the severity of the situation with regards to resistance. Four-fold increases in cisplatin concentration would be required for these cells; this is impractical due to well-known toxicity problems with cisplatin. As such, new treatments are urgently required to combat resistance; this research provides the starting point for further research into the possible development of an anticancer agent from *FE*.

FEBH2, with inactivity displayed under the studied conditions was observed to contain 95% bergapten (data not shown), which has a very similar structure to psoralen (Chunyan *et al.*, 2009). Psoralen is known to exhibit phototoxicity (Chunyan *et al.*, 2009), with applications in the treatment of skin disorders, such as psoriasis, when used in combination with UVA. It would therefore, be reasonable to infer that bergapten may also exhibit photo toxicity. Research by two other groups displayed anti-proliferative actions of bergapten in breast cancer cells (Panno *et al.*, 2012, Panno *et al.*, 2010). Bergapten may have applications in the treatment of diseases other than cancer, and future research could evaluate such effects

All **FB1** fractions were observed to inhibit A2780 cell growth; however, there were no statistically significant differences between the fractions. It may be the case that the FB1 fractions' activity is due to one single component or variations of a component present in all of the fractions to varying degrees. Whilst no statistical significance was displayed, there is still the possibility that the differences in IC_{50} values may be of clinical significance. Exploration of other uses for *FE* extracts could also be a direction for further research. Initial investigations of other uses have been carried out, with *FE* extracts displaying antimicrobial activity against certain micro-organisms (Dongfack *et al.*, 2012).

With regards to toxicity effects on normal dividing cells, some work has already been carried out in this area. Sowemimo *et al.*, (2007) observed a lack of toxicity when *FE* leaf extracts were tested in two animal models. In addition, short-term extract administration was found to be safe, with no effects on haematological parameters, body weight or temperature (Bafor and Igbinuwen, 2009).

Results observed with the **MFF1** extract provides a scope for further research; with focus on the reasons for cell growth enhancement. Reasons for this are yet unknown.

5.4 Conclusions

In conclusion it has been shown that the stem bark of *F. exasperata* contains fatty acids that exerts potent inhibitory effects on A2780 OC cells, in particular, The hexane extracts of the leaves also exhibits similar actvity. *F. exasperata* therefore shows significant potential as a cytotoxic agent.

Acknowledgements

5. References

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