Changes in the Protein Profile of Cervical Cancer Mice Xenograft Model in Response to *Streblus asper* Treatment

Muhammad Nabil¹,²*, Azman Seeni¹, Wan Ismahanisa Ismail², Mohd Hafiz Mail³ and Nurhidayah Ab. Rahim²

¹Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia; nabilfikrimail@gmail.com
²Faculty of Health Science, Universiti Teknologi MARA, Cawangan Pulau Pinang, Kampus Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia
³Malaysian Institute of Pharmaceuticals and Neutraceuticals, National Institute of Biotechnology Malaysia

Abstract

Cervical cancer is the third most prevalent cancer in females (2018) with an estimation of 569,847 incidences and 311,365 deaths worldwide despite the rapid advancement of current technology in treating cervical cancer. Radiotherapy and chemotherapy pose side effects and subsequently hinder treatment efficacy. Therefore, taken together with the previous reports of the plants’ ability in treating cancers, *Streblus asper* is suggested to be a potential candidate for cervical cancer. This study was conducted to investigate the anti-cervical cancer potential of *Streblus asper* through the identification of key proteins and their expression that are regulated in the treatment using mice xenograft model. By employing the use of Liquid Chromatography Mass Spectrometry (LCMS), several proteins associated with cancer growth mechanisms were successfully identified. Four-hundred and fifty-two proteins common to both groups were identified, and 122 proteins were found able to be quantified. Among those proteins, 52 proteins were expressed more than 2-fold changes and 12 proteins were selected based on its established relationship with cancers, including annexin A2, 14-3-3 protein, transgelin-2, galectin-1, keratin, heat shock protein 10 and 70, glucose regulated protein (78kDa), gelsolin, alpha enolase, cofilin-1, vimentin, and calreticulin. All these proteins were downregulated upon treatment of cervical cancer tumour by *Streblus asper*. Pathway enrichment analysis revealed 40 related pathways which include among others, metabolism of protein, post-translational protein modification, cellular responses to external stimuli and stress, cell cycle, and apoptosis. These analyses may improve our molecular insight of the mechanisms involved in the treatment of cervical cancer tumour by *Streblus asper* extract.

Keywords: Anticancer, Cervical Cancer, *In vivo*, Proteomics, *Streblus asper*, Xenograft

1. Introduction

With an estimation of 569,847 incidences and 311,365 deaths in 2018 worldwide, cervical cancer is the third most prevalent cancer in females¹. In Malaysia alone, about 1,682 new cases were recorded in the same year¹. Histologically, there are two subtypes of cervical cancer: Squamous Cell Carcinoma (SCC) and adenocarcinoma. Ninety percent of all cervical cancer cases are SCC while adenocarcinoma constitutes another 10% of the cases². Adenocarcinomas are associated with a poorer prognosis compared to SCC³. The existing preventive measures that have been established for cervical cancer are vaccination and precancerous screening.

The most significant contributor to cervical cancer is Human Papillomavirus (HPV)⁴. With over 80 types of well-categorized HPVs, type 16 and 18 have been seen responsible for about 70% of cervical cancer cases...
cases. In response to this, vaccines were developed and used in approximately 70 countries across the globe. However, weaknesses within its implementation such as unbearable cost and lack of knowledge on HPV vaccination have been found to hold back its effectiveness especially in low and middle-income countries. Similar challenges are faced during the precancerous screening strategy upon its introduction. Precancerous screening which is also known as Pap smear seems to have several unique challenges as identified by previous studies, including failure to identify the eligible population, difficulties in access that leads to demotivation of participation, weakness in screening operation, insufficiency in monitoring and follow-up upon non-responders, and inadequacy of systematic monitoring of treatment.

Despite current advancement, the ability to treat cervical cancer and provide complete remission on advanced cases is still limited. The unavoidable side effects of radiotherapy and chemotherapy have been the major setbacks that hinder treatment efficacy. Radiotherapy is often associated with urologic complications which include radiation cystitis, lower urinary tract dysfunction, stricture disease, fistula formation, and the development of second primary cancer. While chemotherapy is commonly linked to toxicities, it often leads to an increased risk of ovarian dysfunction in the elderly. The anti-angiogenic agent can produce adverse effects like hypertension, left ventricular dysfunction and congestive heart failure, acute vascular event, and bleeding tendencies.

Streblus asper, from the family of Moraceae, is a tree that mostly occurs in tropical countries, including Sri Lanka, Malaysia, Thailand, the Philippines, and India. Traditionally, Streblus asper is renowned for its potential in treating various kinds of maladies such as ulcers, epilepsy, obesity, toothache, fever, dysentery, diarrhea, and stomachache. Streblus asper extract has been found to possess anti-fungal, anti-filarial, anti-microbial, anti-viral, anti-inflammatory, anti-hyperglycemic, and anti-diabetic properties. On top of that, many previous findings have proved its ability to inhibit cancers like mouse lymphocytic leukaemia (P388 cells), osteosarcoma (HOS cells), tongue carcinoma (SCC-15 cells), and human nasopharyngeal epidermoid carcinoma (KB cells).

In our previous study, we have successfully revealed the modulation of protein expression upon Streblus asper treatment in HeLa cervical cancer cells. Streblus asper has been found to regulate several important key proteins in HeLa cells growth. These include galectin-1, 10 kDa heat shock protein, dermcidin, keratin, type I cytoskeletal 9, tropomyosin, myristoylated alanine-rich C-kinase, tumour protein D52, folate receptor alpha, and parathyromosin. In addition, it was also found that Streblus asper likely to induce apoptosis via Tumour Necrosis Factor (TNF) signalling.

Unlike the past, the present study employs the use of in vivo model to investigate the capability of Streblus asper to exhibit positive effects on cervical cancer tumour as it does in vitro study. The xenograft model on immunocompromised animals becomes a model of choice to establish tumour growth for protein analysis because it is deemed as more cost effective than genetically engineered animal and is much safer in terms of handling procedure than using carcinogen. This immunocompromised animal is a type of non-obese diabetic (NOD) and severe combined immunodeficient (SCID) mice which are known to have impaired T- and B-cell development, deficiency of natural killer (NK) cell function, reduced levels of activated macrophage, an absence of haemolytic complement, and abnormal function of dendritic cell. These weaknesses increase the engraftment ability of the model for solid transplantable human cancers.

2. Materials and Methods

2.1 Plant Extract

The Streblus asper plant was purchased from a nursery in Tasek Gelugor, Penang. The plant was authenticated by the Malaysian Institute of Pharmaceuticals and Nutraceuticals (FRIM394(T)/TU490/5/1[Sub1] KH). The roots were left to dry for 2 weeks at room temperature. Then, the roots were ground into powder. The powder sample was boiled in distilled water for 30 minutes. The solution was filtered using a 0.75mm filter and freeze-dried the filtered solution. Upon usage, the weight of the freeze-dried powder was measured accordingly and diluted with double deionized distilled water.
2.2 Animal Protocol

The Animal Ethics Committee of Universiti Sains Malaysia (USM) (ref. USM/Animal Ethics Approval/2015/682) granted ethical approval on 7th October 2015. Ten female mice of type Severe Combined Immunodeficient (SCID) aged 5 to 6 weeks were purchased from Prima Nexus Sdn. Bhd. Health certificate was supplemented along with all purchased mice. Five of the mice were grouped as untreated. While another five mice were grouped as treated. We carefully placed all mice in individually ventilated cage (IVC) system with autoclaved woodchips for bedding and controlled conditions of 22±2°C temperature, 55±10% humidity and 12h light/dark lighting. All mice had unrestricted access to sterilized food and water. Upon arrival, all mice were undergoing acclimatization process for 2 weeks. At week 3, HeLa cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 1% sodium pyruvate and 1% penicillin-streptomycin, and incubated in 37°C humidified CO2 incubator, with 5 % CO2 and 95% of air. The medium was replaced every 72 hours until it reached 80% to 90% of cell confluency before sub-culturing was done. Three to ten of cell passages were used in the experiment. The cells were sub-cultured in the ratio of 1:6 in T-25 flask. Cells were seeded and prepared with a concentration of 1x10^7 cells for animal usage. Then, it was injected at the right flank of mice subcutaneously using the restraining method. We followed the US guidelines (NIH publication 85-23, revised in 1985) for laboratory animal use and care while performing the xenograft model. The untreated group was left to live freely without any treatment given. As for the treated group, *Streblus asper* treatment at a dose of 200 mg/kg of body weight was given at week 14 onwards in an approximately 0.4 ml of sterilized water. The treatment was administered through oral gavage on the alternate day. We determined the dosage following the guidelines by OECD (organization of economic corporation and development's) as detailed by Oghenesuvwe 30. The mice’s weight, water and food consumption were monitored and recorded every three days. Any clinical signs and symptoms of health-related problems such as respiratory, skin and growth problems were carefully monitored. Any observed endpoint signs like weight loss exceeding 20% of the body weight, ulceration or infection at the tumour site, and localized tumour invading the surrounding tissues would subject the mice to early euthanasia. The post-mortem was performed on both groups at week 16. All mice were sacrificed using carbon dioxide gas. The harvested tumour tissues were kept in liquid nitrogen for protein analysis.

2.3 Protein Digestion

Tissues collected during post-mortem were kept in liquid nitrogen and cut into smaller pieces. Then, the tissues were mixed up with 500ul of prepared RIPA buffer (1ml of RIPA buffer with 9 ml of double deionized distilled water) in falcon tubes. The tissues were homogenised and sonicated for 60 minutes in ice. Lysed tissues were centrifuged at 15000rpm for 2 minutes and collected the supernatant into a new microtube regarded as a sample tube. Eighty percent cold acetone was added six times of the sample volume to the sample tube. The tube three was inverted three times and incubated at −20°C overnight. Then, the sample tubes were centrifuged at 6000x g for 10 minutes, decanted the acetone, and dried the pellet in a speed vacuum. The pellet was re-suspended in ammonium bicarbonate 50 mM, pH 8.0. The tissue lysates were stored at -80°C prior to further analysis and peptide digestion.

2.4 Peptide Digestion

Samples with total protein of 100 ug were resuspended in 100 ul of ammonium bicarbonate (NH4HCO3). Each sample was added with 100 ul of 0.05% Rapigest™SF. Samples were mixed using Vortex. Then the Vivaspin column MWCO 3000 was used to make the samples more concentrated to a volume of 100 μl. The samples were centrifuged at 14,000 rpm (20800 x g) for 10 – 15 minutes and heated on a thermomixer at 80°C for 15 minutes. Then to each mixture 5 μl of 100 mM DTT was added and incubated them at 37°C for 30 minutes in a thermomixer. Then the samples were added with 5 μl of 200 mM Iodoacetamide and incubated at room temperature for 45 minutes. 5 μl trypsin (0.2 μg/μl) was added to the reaction and incubated each mixture overnight at 37°C. The trypsin digestion reaction was inhibited by adding 1μl of concentrated trifluoroacetic acid (TFA), incubated at 37°C for 20 minutes and centrifuged the mixtures at 14,000 rpm for 10 minutes.
The supernatants were stored at -80°C prior to LCMS analysis.

2.5 LCMS Analysis

LCMS analysis was conducted by using Orbitrap Fusion mass spectrometer coupled with Dionex 3000 Ultimate RSLCnano (Thermo Fisher Scientific) liquid chromatography system. We used the EASY-Spray Column Acclaim PepMap™ C18 (100 A0, 2 µm particle size, 50 µm id x 15 cm) as the analytical column and the Easy column C18 (2 cm, 0.1 mm i.d., 5 µm) as the pre-column. Pre-column was calibrated at a flow rate of 3 ul/min for 15 µl and the analytical column at a flow rate of 250 nL/min for 4 µl, and set the column temperature at 35°C. Then, chromatographically separated 2 µl of samples was injected to the column at a flow rate of 250 nL/min. 0.1% formic acid in deionized water, and 0.1% formic acid in acetonitrile was used as the running buffers. The peptides were eluted by using the following gradient: 91 min at 5 - 40% solvent B, 2 min at 95% solvent B, 6 min at 95% solvent B, and back to 5% solvent B in 2 min. The instrument was operated in the data-dependent mode. The full-scan spectra were collected by using the Orbitrap MS (OTMS); the parameters were: scan range of 310–1800 m/z, resolving power of 120000, AGC target of 4.0 e5 (400 000), and maximum injection time of 50ms. This method consisted of 3 sec Top Speed Mode where precursors were selected for a maximum of 3 sec cycle and we only further analysed precursors with an assigned monoisotopic m/z and a charge state of 2–7 for MS2. We filtered all precursors using a 20 sec dynamic exclusion window and intensity threshold of 5000 and analysed the MS2 spectra by ion trap MS (ITMS) using the following parameters: rapid scan rate with a resolving power of 60000, AGC target of 1.0e2 (100), 1.6 m/z isolation window, and a maximum injection time of 250ms. The precursors were fragmented by using Collision Induced Dissociation (CID) and high-energy collision dissociation (HCD) at normalised collision energy of 30% and 28%. Each sample was analysed thrice.

2.6. Peptide Identification

The result of protein analysis produced by LCMS was in the form of MS/MS raw data. This data was used to make peptide identification. We carried out the process of peptide identification using Thermo Scientific™ Proteome Discoverer™ Software Version 2.1. We improved the vast quality of this data prior to de novo sequencing which employs the peptide sequence from the tandem mass spectrum without the use of sequence database. Based on the peptide sequence obtained, we searched the database, assisted by the Proteome Discoverer™ which was built-in with a result validation using an enhanced target-decoy approach to estimate the False Discovery Rate (FDR), calculated as the percentage of positive hits in the decoy database versus the target database both for proteins and peptides using a decoy fusion approach to ensure that only valid results were reported. Uniprot_ homo sapiens database was downloaded on 27th October 2017 in the database matching against peptide sequence obtained and the following constraints for the searches and filters: carbamidomethylation and oxidation were set as fixed and variable modifications, respectively; maximum missed cleavage was set as two; a maximum of one nonspecific cleavage site of trypsin cleavage; mass tolerance of 0.1 Da for-parent ions and 0.8 Da for MS/MS fragment ions; FDR was set at < 1%; and significant score (-10 lgP) for protein was set as ≥ 20 with at least one unique peptide. The label-free quantification method was used based on the relative intensities of peptide features detected in multiple samples by matching the retention time and mass over charge ratio (m/z). The high-performance retention time alignment algorithm reliably aligned the features of the same peptide from different samples. Mass error tolerance and retention time shift tolerance was set at 0.2 Da and 1 minute, respectively.

2.7. Bioinformatic Analysis

We carried out the analysis of the peptide identified from raw data using Thermo Scientific™ Proteome Discoverer™, leading to the production of protein information from different forms like peptide-spectrum matches (PSM), peptide group, protein groups and proteins. These data were further analysed using three types of bioinformatics tools, including Perseus, Panther and Reactome. Perseus provided a list of protein with its expression and its Uniprot ID. Panther (Protein Analysis Through Evolutionary Relationships) software Version 13.0 (http://pantherb.org/) on the
other hand classified the proteins based on their families and their functionally related subfamilies. The biological function, cellular localisation, and molecular function of identified protein were obtained using this tool. The next tool used was Reactome version 64 (http://reactome.org). This tool was used to reveal comprehensive analysis and interpretation of pathway information. Significant pathways were chosen from the analysis according to the nature of the pathways and its relationship with cancers.

3. Results

3.1 Protein Identification

34317 peptide-spectrum matches (PSM), 2909 peptide group, 639 protein groups and 2058 proteins were identified at the same FDR setting in the untreated samples; and 31938 PSM, 2401 peptide group, 601 protein groups and 1577 proteins in the *Streblus asper* treated samples (Figure 1).

As shown in Figure 2A, based on their biological process, most of the proteins in untreated group were involved in the cellular process (31.9%), followed by metabolic process (27.0%), cellular component organization or biogenesis (13.5%), localization (7.1%), biological regulation (5.3%), response to stimulus (4.9%), developmental process (3.6%), multicellular organismal process (3.1%), biological adhesion (1.4%), immune system process (1.2%), locomotion (0.7%), reproduction (0.2%). Few of them were involved in growth (0.1%). In the treated group, most of the protein was involved in cellular process (30.5%), followed by metabolic process (26.0%), cellular component organization or biogenesis (13.3%), localization (6.9%), biological regulation (5.8%), response to stimulus (5.7%), developmental process (4.6%), multicellular organismal process (3.3%), biological adhesion (1.7%), immune system process (1.1%), locomotion (0.8%) and reproduction (0.2%). Few of them were involved in growth (0.1%).

As shown in Figure 2B, based on their molecular function, most of the proteins in the untreated group were involved in binding activity (38.1%), followed by catalytic activity (36.8%), structural molecule activity (16.1%), transporter activity (2.6%), antioxidant activity (2.2%), translation regulator activity (2.0%), receptor activity (1.3%). The least group of protein were involved in signal transducer activity (0.9%). In the treated group, most of the proteins were involved in binding activity (40.7%), followed by catalytic activity (37.0%), structural molecule activity (12.8%), antioxidant activity (2.5%), translation regulator activity (1.9%), signal transducer activity (1.7%) and transporter activity (1.7%). Few of the proteins were involved in receptor activity (1.6%).

As shown in Figure 2C, based on their cellular component, most of the proteins in untreated group were involved in cell part (40.4%), followed by organelle (29.4%), macromolecular complex (19.7%), membrane (5.7%), extracellular region (2.6%), extracellular matrix (1.1%) and cell junction (0.6%). Few of the proteins were involved in synapose (0.4%). In the treated group, most of the proteins were involved in cell part (40.4%), followed by organelle (27.9%), macromolecular complex (19.5%), membrane (7.1%), extracellular region (2.9%) and extracellular matrix (1.4%). Few of
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3.2. Protein Quantification

Only 452 proteins were common to both groups when compared to the 639 proteins identified in the untreated group, and 601 proteins identified in *Streblus asper* treated group (as illustrated in Figure 3). We further analysed these identified proteins using Perseus to identify the differentially expressed proteins in both groups. The log₂ ratios of normalised volume obtained from the Thermo Scientific™ Proteome Discoverer™ Software Version 2.1 represented the quantitative variations in protein abundance per injection between the protein group of the untreated and treated samples.

![Figure 2. GO analysis illustrates classes of proteins differing between untreated and treated tumour tissues. Protein with significant differences between the two groups was subjected to GO classification in terms of (A) biological process, (B) molecular function, and (C) cellular localisation.](image)

3.3. Pathways Analysis

Pathway enrichment analysis was carried out on common proteins with at least two peptides found in untreated and treated samples. The analysis was done using Reactome, an online bioinformatics tool. From that analysis, 40 pathways that played a meaningful role in cancer were selected (as illustrated in Table 2).

4. Discussion

The present study focused on differentially expressed proteins that were discovered based on a comparison of protein expression between *Streblus asper* treated and untreated groups. Throughout the study protocol, no mice were subjected to early euthanasia due to reaching the signs of endpoint. Of the 1240 proteins identified from both groups, 452 proteins (57.4%) were common...
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Changes in the Protein Profile of Cervical Cancer Mice Xenograft Model in Response to Streblus asper Treatment

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<td>TP53 Regulates Metabolic Genes</td>
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or identical to both groups. Among these common proteins, only 52 of them were differentially expressed. Twelve of them (including those derived from the same family) were selected based on their known establishment and relationship with cancers, including annexin A2, 14-3-3 protein, transgelin-2, galectin-1, keratin, heat shock protein, glucose regulated protein (78kDa), gelsolin, alpha enolase, cofilin-1, vimentin, and calreticulin. All these proteins were found downregulated by at least 2-fold in cervical cancer tumour tissue upon treatment with *Streblus asper*.

Annexin A2, found in various types of tumour cells, is a cytoskeletal protein produced from 40kb gene on the long arm of chromosome 15. It is overexpressed in cancers like breast, liver, prostate, and pancreas and involved in cancers via signal transduction, angiogenesis, tumour invasion, metastasis, and apoptosis. The upregulation of annexin A2 expression occurs in cervical carcinoma, explaining how *Streblus asper* treatment was capable of downregulating its expression in cervical cancer according to our findings.

The 14-3-3 protein is a phosphoserine/phosphothreonine binding protein consisting of 7 isoforms (β, γ, ε, η, ζ, σ and τ/θ) and imperative in many cellular processes, including cell cycle progression, DNA repair, apoptosis inhibition, cell differentiation and senescence control, and cell adhesion and motility regulation. Many studies have indicated that increasing 14-3-3 protein expression enhances cancer growth. For instance, the increased expression of 14-3-3 sigma, theta, and epsilon isoforms promote cell survival, invasion and metastasis in prostate, breast, and liver cancer cells respectively. As for beta isoform, the increased expression is associated with progression of liver cancer. The suppression of 14-3-3 zeta expression leads to inhibition of lung cancer cells growth. However, few isoforms of 14-3-3 proteins have been characterised in cervical cancer.

Transgelin-2, a member of actin-binding protein, is imperative in cell differentiation, apoptosis, proliferation, and migration. It regulates the cytoskeletal RNA transcription and processing, chromatin remodelling, and nuclear transport. The expression of transgelin-2 is higher in tumour tissue in comparison to healthy tissue. Its overexpression is associated with tumorigenesis. Besides that, the elevation of its expression is proportionate to the tumour progression. Thus, it was previously suggested
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As a tumour biomarker, transgelin-2 was observed to be highly expressed in cervical SCC while its suppression inhibits cell migration and secretion of matrix metalloproteinases. We found that cervical cancer tissue treated with *Streblus asper* resulted in the downregulation of transgelin-2 expression compared to the untreated cancerous tissues. Based on the previous findings, transgelin-2 is likely to possess a different set of expression levels in tumourigenesis and malignancy transformation.

Galectins are group of proteins that occur in different subtypes (1,2,3,4,5,7,8,9,10,11,12,13,14 and 15). These subtypes are determined according to their structure which comprises of tandem, dimeric and chimeric. Galectins are able to bind β-galactoside sugar. In breast cancer, galectin-1 has been found contributing to disease progression and metastasis. Besides that, it also contributes to the progression of prostate cancer. A study has associated the overexpression of galectin-1 with poor prognosis of lung cancer. The invasiveness of cervical cancer has been observed to correspond with the expression of galectin-1. Based on this, *Streblus asper* seems to be effective against cervical cancer by downregulating the expression of galectin-1.

Keratins are the structural proteins of the epithelial cells which line multiple organs and cavities in the body. Keratins play a crucial role in the determination of cell size, control of the translational process, cell proliferation, cell type-specific, transportation of organelle, transformation of malignancy and various stress responses. Keratins are expressed in cancers like breast, cervix, colon, liver, kidney, lung, pancreas, prostate. Given its functional roles in normal epithelial cells, keratins are also actively involved in cancer cell progression, metastasis, and response to treatments. Keratin 18 has been found to be overexpressed in breast cancer and correlated with poor prognosis of the disease. In the present study, *Streblus asper* exhibited downregulation of keratin 18 in cervical cancer. Therefore, it is suggested that *Streblus asper* could contribute to the improvement of cervical cancer prognosis.

Heat shock proteins (HSPs) are a protein family that facilitates protein protection against degradation, hypoxia, thermal destruction and oxidative stress. HSPs are present in different types depending on their relative molecular sizes, including HSP27, HSP40, HSP60, HSP70, HSP90, and family of large HSPs (HSP110 and HSP170). In cancer cells, HSPs protect the oncoprotein associated with proliferation, differentiation, and progression. HSP10, a chaperone protein located in the mitochondria, is overexpressed in tissues with carcinogenesis. This has been discovered in excocervical carcinoma, large bowel, and serous ovarian carcinoma. HSP70 is critical in the inhibition of apoptosis. It facilitates the inhibition of caspases activity and blockage of TNF alpha mediated apoptosis. *Streblus asper* treatment seems capable of downregulating the expression of HSP10 and HSP70, potentially leading to suppression of carcinogenesis and induction of apoptosis. Nevertheless, the detailed explanation for this remains underexplored.

Glucose-regulated protein 78kDa (GRP78), a stress-inducible molecular chaperone, usually resides in the endoplasmic reticulum (ER). The endoplasmic reticulum-GRP plays significant roles in protein folding, secretion, degradation, apoptosis suppressor, calcium homeostasis, cytokine and chemokine secretion, and ER signalling control and ER structural integrity. It has been proven to be elevated in various cancer cells. In the xenograft tumour model, knockdown of GRP78 resulted in the metastasis process being inhibited. Meanwhile, *in vitro* analysis revealed that the knockdown of GRP78 suppresses cell invasion. Consistent with our data, *Streblus asper* treatment may cause inhibition of cervical cancer tumour by downregulating the expression of GRP78.

Gelsolin is another actin-binding protein crucial in numerous activities pertaining to actin cytoskeletal function, including motility, maintenance of cell morphology and polarity, intracellular trafficking and cell division. The upregulation of this protein in oral SCC cell line may promote cell proliferation. In other analyses, gelsolin was found overexpressed in the liver cancer cell line (HepG2). The study indicated the relationship between gelsolin and p53 expression whereby the gelsolin might be able to inhibit apoptosis through the p53 suppression. In other words, it negatively regulated the p53 expression. Overexpression of gelsolin was also discovered in cervical cancer cases. This was proven in tissue and plasma samples. The increase was correlated with cellular proliferation and
migration abilities. In accordance with that, our result suggests that *Streblus asper* downregulated the expression of gelsolin in cervical cancer tumour and thus reflected the reduction of tumour proliferation.

Alpha enolase, a glycolytic enzyme responsible for ATP generation in cells, is a candidate marker for cancer prognosis since it promotes cell growth, invasion, and migration in various cancers. In gastric cancer, it promotes cell proliferation and metastasis by modulating AKT signalling cascade. It also regulates similar pathways to encourage cell growth migration and invasion in non-small cell lung cancer. The upregulation of alpha enolase occurs in various tumour cells compared to normal cells. These overexpression patterns suggest that highly proliferative cells require higher glycolytic activity as it is understood in nature to be more aggressive than normal cells.

Cofilin-1 is essential in the regulation of actin filaments. Generally, cofilin expression is upregulated in many types of cancer cells, including lung, pancreatic, breast, oral, renal, ovarian, and liver. Cofilin-1, a member of the cofilin family, is overexpressed in cancers like colorectal, bladder, pancreatic, oral and cervical cancer. The overexpression of cofilin-1 in SCC and adenocarcinoma of the gallbladder is correlated to the disease progression. Furthermore, it promotes cell growth, proliferation, cell cycle progression, migration, invasion, adhesion as well as inhibiting apoptosis. Concordant with our data, cofilin-1 expression was initially high in untreated cervical cancer and it decreased upon treatment with *Streblus asper*. However, the underlying mechanism of cofilin-1 in cancer is yet to be determined.

Vimentin is involved in maintaining cellular integrity and providing resistance against stress. It is an intermediate filament family of protein that serves as a potential biomarker for cancers. Studies have shown that vimentin expression is upregulated in many kinds of cancer cells like prostate, gastric, colorectal, pancreatic, oesophageal, breast, and cervical cancers. Most of the studies showed that the expression of vimentin is highly associated with cancer invasiveness. The suppression of vimentin leads to a reduction in cancer invasion potential. In our study, *Streblus asper* treatment seems to be effective against cervical cancer tumour as it downregulates vimentin expression by more than 2-fold changes.

*Streblus asper* downregulates the expression of calreticulin in cervical cancer, indicating the positive effects of *Streblus asper* on cervical cancer since the overexpression of calreticulin enhances proliferation and metastasis of cancers. Calreticulin is overexpressed in cancers like oral, breast, pancreatic, oesophageal, gastric, colorectal, and bladder. On the contrary, the knockdown of calreticulin resulted in the inhibition of cancer proliferation and metastasis. As discussed by Lu and co-researchers (2015), calreticulin plays multiple roles in cell physiological and pathological responses. Its main functions include homeostasis of calcium ion and protein chaperoning. However, the possible relationships between its roles and cancer mechanisms especially proliferation and metastasis remain elusive.

5. Conclusion

In this study, several proteins have been identified from the *Streblus asper* treated and untreated cervical cancer tumour tissues. Twelve proteins were selected as they have been reported to have relationships directly or indirectly with cancers. Of these, twelve of the proteins were observed to support our hypothesis while two seem to be contradictory. Based on these, it is likely that *Streblus asper* demonstrated anticancer activities on cervical cancer tumour tissue in vivo through the downregulation of annexin A2, 14-3-3 protein, transgelin-2, galectin-1, keratin, heat shock protein 10 and 70, glucose regulated protein (78kDa), gelsolin, alpha enolase, cofilin-1, vimentin, and calreticulin. Hence, further validation of the profile and expression pattern will significantly contribute to the establishment of therapeutic biomarker of cervical cancer targeted by *Streblus asper*. Besides that, it will also provide a comprehensive understanding of the *Streblus asper* effects at cellular and molecular levels.

6. Conflict of Interest Statement

The authors declare that there are no conflicts of interest.
7. Acknowledgement

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8. References


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