EVALUATING CYTOTOXICITY POTENCY OF GLUCOMORINGIN FROM THE SEEDS OF Moringa oleifera Lam AGAINST PROSTATE CANCER CELL LINE, PC-3

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By

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Inhibition of several protein pathways involved in cancer cell regulation is a necessary key in the discovery of cancer chemotherapy. *Moringa oleifera* Lam is often used in traditional medicine in several countries for the treatment of various illnesses. The plant was also found to contain bioactive compounds with therapeutic potential against various cancer cells. This study was carried out to determine the cancer cell inhibition of compounds from natural origins, specifically from the seeds of *M. oleifera*, where glucomoringin can be found abundantly and might carry the potential to inhibit the proliferation of prostate cancer cells.

The crude seed extracts were obtained from water, acetone, chloroform, ethanol, methanol, and hexane. The water extract produced the highest crude yield (21.78%). The crude extracts were initially screened for phytochemical content, followed by cell viability tests using MTT assay against MCF 7, HepG2, DU 145, and PC-3 cell lines. Crude hexane, acetone and chloroform, were found to have coumarin and fat. Crude ethanol contains coumarin, quinone and fat. Crude methanol contains only saponin, coumarin and quinone, and finally, crude water contains saponin, alkaloid and coumarin. The crude water extract showed an inhibition (IC_{50}) value with a time-dependent manner at 24, 48, and 72 hours of treatment. The crude water extract showed low IC_{50} against MCF 7 (39.95 µg/ml), Hep G2 (20.9 µg/ml), DU 145 (32.12 µg/ml), and PC-3 (4.12 µg/ml) at 72 hours respectively. The crude water extract was chosen to be use in the experiment because it was the most effective against the cell line that shows the lowest IC_{50} value as well as giving the highest crude yield All IC_{50} values are significant compared with control after analysis using one sample T-test ($P < 0.05$)

The crude water extract was used for further for compound isolation, which yielded 9.43% of glucomoringin (GMG). Subsequently, the compound was

activated with myrosinase enzymes to yield its active compound, glucomoringin isothiocyanate (GMG-ITC), followed by characterization using high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopy analyses. The isolated GMG-ITC was screened for its cytotoxicity, employing MTT assays against all the cell lines mentioned previously, and PC-3 was found to be more selective with IC_{50} of the isolated compound is 3.5 µg/ml. All IC_{50} values are significant compared to control after analysis using one sample T-test ($P < 0.05$). The IC_{50} value of GMG-ITC was then used in the following studies, including morphological observation of apoptosis via AO/PI and TUNEL assays on the PC-3 cell line. The cell showed clear apoptotic body characterisation under phase contrast analysis, as well as colour changes with membrane blebbing and chromatin condensation in the AO/PI staining, followed by dark brown colour staining in the TUNEL assay due to nuclear DNA fragmentation of the cancer cells.

The cytotoxic potency study was further performed with Annexin V-FITC, cell cycle analysis, mRNA quantification, and protein signalling. In Annexin V-FITC, the apoptosis cell activity increased significantly ($P < 0.05$) from 24, 48, to 72 hours of treatment compared to the control by GMG-ITC. The apoptotic cell percentage increased significantly ($P<0.01$) from 21.35% (24 hours), followed by 34.47% (48 hours) and finally 72.9% (72 hours) after treatment. The compound was observed to arrest the cell cycle at G_2/M phase, which is an important phase in apoptosis. mRNA quantification indicates Nrf2 expression was downregulated significantly ($P<0.05$), but p53, Bax, and parp 6 were upregulated by the treatment of the GMG-ITC against PC-3 cell lines. The expression of p53 can be linked with a blockage of cell cycle progression at G_2/M phase and the upregulation of Bax, caspase-3, while Bcl2 and Nrf2 were downregulated. Finally, protein analysis employing cell signalling immunoassay studies by Multiplex analysis revealed the modulation of apoptotic proteins after the treatment of the cancer cells with GMG-ITC. The expression of proteins in the analysis; JNK, Bad, Bcl2, and p53 were significantly upregulated ($p<0.01$) compared to control. It indicates that early apoptosis expression was triggered by the protein signalling induced by the treatment. In this study, apoptosis induction and DNA fragmentation processes were observed in treated PC-3 cells. These phenomena suggest that the bioactive compound from *M. oleifera* seed could be useful for future cytotoxic agent against prostate cancer.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENILAIAN POTENSI SITOTOKSISITI OLEH GLUKOMORINGIN DARI BIJI *M. oleifera* Lam TERHADAP SEL SELANJAR KANSER PROSTAT PC-3

Oleh

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Perencatan beberapa laluan protein yang terlibat dalam pengawal aturan sel kanser adalah kunci keperluan dalam penemuan kemoterapi kanser. *Moringa oleifera* sering digunakan dalam ubatan tradisional oleh beberapa negara dalam rawatan beberapa penyakit. Tumbuhan ini juga didapati mengandungi sebatian bioaktif dengan potensi terapeutik terhadap pelbagai sel kanser. Kajian ini dijalankan untuk menentukan penghalang sel kanser yang berkesan daripada unsur semula jadi, khususnya biji benih *M. oleifera* di mana glukomoringin boleh didapati dengan banyaknya yang mungkin membawa potensi untuk menghalang percambahan sel kanser prostat. Kajian ini dilakukan menggunakan pendekatan *in vitro* dan molekul.

Ekstrak mentah tumbuhan diperoleh daripada air, aseton, kloroform, etanol, metanol dan heksana di mana ekstrak air memberikan hasil ekstrak tertinggi (21.78%). Ekstrak mentah pada mulanya disaring untuk kandungan fitokimia dan viabiliti sel menggunakan ujian MTT terhadap MCF 7, HepG2, DU 145 dan sel PC 3. Ekstrak heksana, aseton dan kloroform didapati mengandungi kumarin dan lemak. Ekstrak etanol mengandungi kumarin, quinon dan lemak. Ekstrak metanol hanya mengandungi saponin, kumarin dan kuinon dan terakhir sekali, ekstrak air mengandungi saponin, alkaloid dan kumarin. Ekstrak air mentah menunjukkan nilai perencatan yang stabil (IC_{50}) dengan kadar berturutan pada masa 24, 48 dan 72 jam rawatan. Ekstrak itu menunjukkan nilai IC_{50} rendah terhadap MCF 7 (39.95 µg / ml), Hep G2 (20.9 µg / ml), DU 145 (32.12 µg / ml) dan PC 3 (4.12 µg / ml) pada 72 jam. Semua nilai IC_{50} adalah signifikan selepas dianalisis menggunakan satu sampel T-test ($P < 0.05$)

Ekstrak air mentah digunakan untuk kajian selanjutnya yang melibatkan penghasilan ekstrak, 21.78% dan pengasingan kompaun yang menghasilkan 9.43% glukomoringin (GMG). Kompaun telah diaktifkan dengan enzim myrosinase untuk menghasilkan kompaun aktif, glukomoringin isotiosianat (GMG-ITC) diikuti oleh pencirian menggunakan analisis kromatografi cecair prestasi tinggi (HPLC) dan analisis spektroskopi resonans magnetik nuklear (NMR)

Kemudian GMG-ITC yang terisolasi telah digunakan untuk kajian sitotoksisitinya menggunakan ujian asai MTT terhadap semua sel-sel yang telah disebutkan dan PC 3 didapati paling selektif dengan IC_{50} (3.5 µg / mL). Kesemua nilai IC_{50} adalah signifikan selepas dianalisis menggunakan satu sampel T-test ($P < 0.05$).Nilai IC_{50} GMG-ITC kemudiannya digunakan dalam kajian berikut termasuk pemerhatian morfologi apoptosis melalui ujian AO/PI dan TUNEL pada sel PC 3. Sel ini menunjukkan ciri-ciri tubuh apoptosis yang jelas di bawah analisis kontras fasa serta perubahan warna dengan membran dan penggumpalan kromatin dalam pewarnaan AOPI diikuti oleh warna coklat gelap di dalam TUNEL asai disebabkan oleh pemecahan DNA nuklear sel kanser.

Kajian potensi sitotoksisiti selanjutnya dilakukan dengan Annexin V-FITC, analisis kitaran sel, kuantifikasi mRNA dan isyarat protein. Dalam kajian Annexin V-FITC, sel-sel apoptosis meningkat dengan ketara ($P<0.05$) dari 24, 48 hingga 72 jam rawatan berbanding dengan sel kawalan oleh GMG-ITC. Peratusan sel apoptotik meningkat dengan ketara ($P<0.001$) dari 21.35% (24 jam), diikuti oleh 34.47% (48 jam) dan akhirnya 72.9% pada (72 jam) rawatan. Kompaun itu didapati menghentikan kitaran sel pada kitaran G_2/M yang merupakan fasa penting dalam apoptosis. Pengkuantifikasi mRNA menunjukkan ekspresi Nrf2 adalah berkurang dengan ketara ($P<0.05$) tetapi p53, Bax dan Parp 6 menjadi meningkat setelah rawatan GMG-ITC dilakukan terhadap sel PC 3. p53 boleh dihubungkan dengan pengurangan perkembangan kitaran sel pada fasa G_2/M dan pengawalan Bax, caspase-3 sementara Bcl2 dan Nrf2 telah direncatkan dengan ketara ($P<0.01$). Akhirnya, analisis protein yang menggunakan kajian immuno asai isyarat sel dilakukan oleh analisis Multiplex mendedahkan modulasi protein apoptotik selepas rawatan sel kanser dengan GMG-ITC. Ekspresi JNK, Bad, Bcl2 dan p53 adalah protein yang dikawal. Ia menunjukkan ekspresi apoptosis awal yang berlaku oleh isyarat protein yang terlibat disebabkan oleh rawatan yang berlaku. Dalam kajian ini, induksi apoptosis dan pemecahan DNA dapat diperhatikan dalam PC 3 yang dirawat. Fenomena dari kajian menunjukkan bahawa sebatian bioaktif dari biji *M. oleifera* boleh digunakan untuk kegunaan kajian sitotoksik terhadap kanser prostat pada masa depan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of doctor of philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ADP	Adenosine 5'-diphosphate
Ala	Alanine
AO/PI	Acridine Orange/Propidium Iodide
Arg	Arginine
bp	Base pair
BP	Base peak
C	Carbon
CDKs	Cyclin-Dependent Kinase
CDCL3	Deuterated chloroform
CGM	Complete growth medium
CO_2	Carbon dioxide
DAPI	4',6-diamidino-2-phenylindole
DHA	Hydrogen donor; hydrogen; hydrogen acceptor
DMSO	Dimethyl sulfoxide
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphates
DU 145	Prostate adenocarcinoma cell line
ELISA	Enzyme-linked immuno assay
FBS	Foetal Bovine serum
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GMG	Glucomoringin
GMG-ITC	Glucomoringin isothiocyanate
GLs	Glucosinolates
Gly	Glycine
GSH	Glutathione
H	Hydrogen
H_2O	Water
HepG2	Human hepatocyte carcinoma cell line
IC_{50}	Inhibitory concentration at 50%
ITC	Isothiocyanate
IU	International unit
Lys	Lysine
MAPK	Mitogen-activated protein kinase
Mg^{2+}	Magnesium ion
mRNA	Messenger RNA
MCF-7	Human breast adenocarcinoma
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl Bromide tetrazolium
MW	Molecular weight
MYR	Myrosinase
NDP	Nucleoside diphosphates
NMPs	Nucleoside monophosphates
NF-κB	Nuclear Factor kappa B
NMR	Nuclear Magnetic Resonance
O_2	Oxygen
PARP	ADP-ribose polymerase

PBS	Phosphate buffered saline
PE	Petroleum ether
Phe	Phenylalanine
PC 3	Human Prostate adenocarcinoma cell line
PCR	Polymerase chain reaction
RLU	Relative Luminescence Unit
ROS	Reactive Oxygen Species
RNA	Ribonucleic acid
RPs	Ribosomal proteins
RPMI	Roswell Park Memorial Institute
rRNA	Ribosomal RNA
RT-PCR	Reverse transcription-polymerase chain reaction
TLC	Thin-layer chromatography
Tris-HCl	Tris-hydrochloride
TdT	Terminal deoxynucleotidyl transferase
TUNEL	Terminal deoxynucleotidyl transferase dUTP labeling nick end
UV	Ultraviolet
WHO	World Health Organisation
Globocan	Global cancer incidence, mortality and prevalence

CHAPTER 1

INTRODUCTION

1.1 Research Background

Cancer can be described as a disease caused by aberrant cells that grows uncontrollably that it evades, erodes, then affects or destroys normal cells or tissues. According to the Malaysian National Cancer Institute (2016), there are about 12 common types of cancer that occurs among citizens nationwide. Five types of cancers that most commonly occur in males (shown in Table 1.1) include colorectal, lung, nasopharynx, lymphoma, and prostate cancer. The disease can attack young children and adults, regardless of race, however, men who are more than 50 years old are more at risk of developing prostate cancer.

Table 1.1: Most common cancers among males in Malaysia

Types of Cancer	Incidence (%)
Colorectal	16.3
Lung	15.8
Nasopharynx	8.1
Lymphoma	6.8
Prostate	6.7

(Source: Malaysian National Cancer Registry Report ,2016)

When a cell grows abnormally, but kept under control by the internal immune system, it may not cause any harm. The cell can be called benign or non-cancerous cells. But when the cell starts to replicate, forming lumps or growths, it will potentially lead to the formation of cancerous or malignant cells. Cancerous cells usually spread to the surrounding tissues, cause cellular destruction, and facilitates the development of other tumors (Chen et al., 2015; Pal et al., 2014). Malignant or cancerous cells can migrate to vascular or lymphatic systems as well as grow anywhere inside the body or organ, thus causing various types of cancers which are life threatening (Roomi et al., 2015).In Malaysia, the number of cancer cases increased by 7.96% within a span of four years (Ministry of Health Malaysia, 2013). It is one of the most common diseases that contribute to the mortality rate among the Malaysian population, with report cases stating 44.6% (8123) in males and 55.4% (1096) in females (Malaysian National Cancer Registry Report, 2016). Cancer has been a global burden in recent years. In cancer treatments, the most common way to treat cancers are through surgery, chemotherapy, and radiation. Most cancer patients will undergo surgery

followed by chemotherapy. Side effects are oftentimes reported by patients undergoing chemotherapy treatment, which include hair loss, fatigue, bruises, and hormonal problems (Zagouri et al., 2015).Medicinal plants that contains phytochemicals such as alkaloid, polyphenols and flavonoids provide a significant contribution in combating cancer. The use of medicinal plants for disease treatment have been reported in the past. Natural plant sources played an important role in discovering alternative medicine that helps prevent cancer. It was reported that around 250,000 plant species are believed to have anti-cancer properties. Medicinal plants are regarded to have a high potential for novel drug discovery, nutraceuticals, and also provides food supplements (Jeeshna and Paulsamy, 2011; Anwar, et al., 2007).The plant compounds, podophyllotoxin led the discovery of cancer treatment drugs for lung cancer and testicular. (Clarke et al., 2008). Paclitaxel and docetaxel were discovered from natural plant compound in Pacific Yew tree used in cancer treatments (Stearns and Wang, 2011). These compounds were derived from plants and are utilized as natural anti-cancer agents in the market. Paclitaxel was reported to have cognitive side effects on cancer patients (Smith et al., 2016). In fact, ovarian cancer survivors were also found to have early menopause, as well as diminished ovarian function which is mainly caused by procarbazine and alkylating agents (Overbeek et al., 2017). Diminished ovarian function can lead to a lot of other effects on the patients.Due to the increasing number of cancer cases, it is important to discover more potential chemotherapeutic agents against cancer. Therefore, it is crucial to study medicinal plants and their natural compounds to counter the lack of natural anticancer treatment drug effectively.

1.2 Problem statements

Glucosinolates, which derived from isothiocyanates (ITC) such as benzyl isothiocyanate (B-ITC), allyl isothiocyanate (AI-TC), and phenylethyl isothiocyanate (PE-ITC), have been reported for their protective effects against cancer cells. The ITCs had shown cytotoxic effects towards breast, liver and lung cancer via modulation of MEK/ERK, PI3K/AKT and Nrf2, intrinsic and extrinsic apoptosis pathway. However, lack of study had been done on glucomoringin isothiocyanate (GMG-ITC) against prostate cancer cell line to determine its cytotoxic effect. Isothiocyanates (ITCs) contains anti-cancer agents that may provide the solution for natural anti-cancer treatment with less side effect for prostate cancer treatment. This study will provide more facts on the cytotoxic potency of glucomoringin isothiocyanate (GMG-ITC) to induce apoptosis in prostate cancer cell and determine the pathways involved to inhibit tumor or cancer progression.

1.3 Justification of Study

Cancer is well known as one of the deadly diseases at present, which it can be categorized as death sentence due its consequences or series of events, and stages, which some cancer patients have to go through. The current treatment did not promise an escape from the devastating disease due to its high cost of treatments and side effects of modern drugs. Cytotoxic compound or therapeutic compound discovered from natural sources are usually highly abundance and readily available. The compound will also be cheap to produce with no or less side effect. Thus, it will benefit the societies either in preventing cancer or treatment of cancer. The discovery of cytotoxic potential of the compound from natural resources may give hope or opportunities in treatment of cancer especially for the most common cancer among the societies.

1.4 Hypothesis

1.4.1 Null Hypothesis

GMG-ITC does not show cytotoxicity potency through the induction of apoptosis and cell cycle arrest in PC-3 cells.

1.4.2 Alternative Hypothesis

GMG-ITC shows cytotoxicity potency through the induction of apoptosis and cell cycle arrest in PC-3 cells.

1.5 Objectives of research

General objective is to evaluate cytotoxic potency of glucomoringin isolated from *Moringa oleifera* Lam against prostate cancer cell line, PC-3

Specific objectives

1. To assess cytotoxic effects of *M. oleifera* Lam crude seeds extract on several cancer cell lines
2. To elucidate glucomoringin isolated from crude water seed extract of M oleifera Lam
3. To evaluate the cytotoxicity potency of glucomoringin through the induction of apoptosis and cell cycle arrest of PC-3 cells.

REFERENCES

Abdelwahab, S. I., Abdul, A. B., Zain, Z. N. M., & Hadi, A. H. A. (2012). Zerumbone inhibits interleukin-6 and induces apoptosis and cell cycle arrest in ovarian and cervical cancer cells. *International Immunopharmacology*, *12*(4), 594–602.

Ah, L., Ming, P., Lee, Y., Ming, W., Tsan, A., Kei, & M., Fai, C. (2017). Bisphenol A and other environmental risk factors for prostate cancer in Hong Kong, E*nvironment International* 107 (April), 1–7.

Ajazuddin, Alexander, A., Qureshi, A., Kumari, L., Vaishnav, P., Sharma, M., Saraf, S. (2014). Role of herbal bioactives as a potential bioavailability enhancer for Active Pharmaceutical Ingredients. *Fitoterapia*, *97*, 1–14.

Akinsulire, O. R., Aibinu, I. E., Adenipekun, T., Adelowotan, T., & Odugbemi, T. (2007). *In Vitro* antimicrobial activity of crude extracts from plants *Bryophyllum Pinnatum* and *Kalanchoe Crenata. African Journal of Traditional Complementary and Alternative Medicines AJTCAM African Networks on Ethnomedicines*, *4*(3), 338–344.

Ali, E. (n.d.). *Moringa oleifera* miracle tree for green technology: a review .Faculty of Chemical and Natural Resources Engineering, University Malaysia Pahang, Lebuhraya Tun Razak, Gambang, 26300 Kuantan, Pahang, Malaysia.

Ali, G. H., El-Taweel, G. E., & Ali, M. A. (2004). The cytotoxicity and antimicrobial efficiency of *Moringa oleifera* seeds extracts. *International Journal of Environmental Studies*, *61*(6), 699–708.

American Cancer Society. (2016). Cancer Facts & Figures 2016. *Cancer Facts & Figures 2016*, 1–9.

Andrade, L. De, Aparecido, F., Alves, R., Henrique, T., Maria, P., Paiva, G., & Barroso, B. (2017). Cytotoxicity of the coagulant *Moringa oleifera* lectin (cMoL) to B16-F10 melanoma cells. *Toxicology in vitro (44)*, 94–99.

Anwar, F., Latif, S., Ashraf, M., & Gilani, A. H. (2007). Moringa oleifera : A food plant with multiple medicinal uses. *Phytotherapy Research 25*(September 2006), 17–25.

Arlt, A., Müerköster, S. S., & Schäfer, H. (2013). Targeting apoptosis pathways in pancreatic cancer. *Cancer Letters*, *332*(2), 346–58.

Barillari, J., Gueyrard, D., Rollin, P., & Iori, R. (2001). Barbarea verna as a source of 2-phenylethyl glucosinolate precursor of cancer chemopreventive phenylethyl isothiocyanate. *Fitoterapia*, *72 2001*, 760–764.

Bennett, R. N., Mellon, F. a, Botting, N. P., Eagles, J., Rosa, E. a. ., & Williamson, G. (2002). Identification of the major glucosinolate (4-mercaptobutyl glucosinolate) in leaves of *Eruca sativa L.* (salad rocket). *Phytochemistry*, *61*(1), 25–30.

Blažević, I., & Mastelić, J. (2009). Glucosinolate degradation products and other bound and free volatiles in the leaves and roots of radish (*Raphanus sativus L*.). *Food Chemistry*, *113*(1), 96–102.

Boghossian, S., & Hawash, A. (2012). Chemoprevention in colorectal cancer where we stand and what we have learned from twenty year's experience. *The Surgeon : Journal of the Royal Colleges of Surgeons of Edinburgh and Ireland*, *10*(1), 43-52.

Boik, J. (2001). Natural compounds in cancer therapy promising nontoxic

antitumor agents from plants and other natural sources (1st Ed). United States of America: *Oregon Medical Press*.

Brunelli, D., Tavecchio, M., Falcioni, C., Frapolli, R., Erba, E., Iori, R., & D'Incalci, M. (2010a). The isothiocyanate produced from glucomoringin inhibits NF-kB and reduces myeloma growth in nude mice in vivo. *Biochemical Pharmacology*, *79*(8), 1141–8.

Cai, Y., Luo, Q., Sun, M., & Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences*, *74*(17), 2157–84.

Carlsson, S., & Vickers, A. (2015). Spotlight on prostate cancer: The latest evidence and current controversies. *BMC Medicine*, *13*(1), 4–6.

Cheenpracha, S., Park, E.J., Yoshida, W. Y., Barit, C., Wall, M., Pezzuto, J. M., & Chang, L. C. (2010). Potential anti-inflammatory phenolic glycosides from the medicinal plant *Moringa oleifera* fruits. *Bioorganic & Medicinal Chemistry*, *18*(17), 6598–602.

Chen, I.-C., Lin, C.-H., Jan, I.-S., Cheng, A.-L., & Lu, Y.-S. (2015). Bevacizumab might potentiate the chemotherapeutic effect in breast cancer patients with leptomeningeal carcinomatosis. *Journal of the Formosan Medical Association*, 1–6.

Chen, R., Ren, S., Yiu, M. K., Fai, N. C., Cheng, W. S., Ian, L. H., Sun, Y. (2014). Prostate cancer in Asia: A collaborative report. *Asian Journal of Urology*, *1*(1), 15–29.

Cheung, K.L., & Kong, A.N. (2010). Molecular targets of dietary phenethyl isothiocyanate and sulforaphane for cancer chemoprevention. *The AAPS Journal*, *12*(1), 87–97.

Chollom S. C (2012). Investigation of aqueous extract of Moringa oleifera lam seed for antiviral activity against newcastle disease virus in ovo. *Journal of Medicinal Plants Research*, *6*(22), 3870–3875.

Chuang, P.H., Lee, C.W., Chou, J.Y., Murugan, M., Shieh, B.J., & Chen, H.M. (2007). Anti-fungal activity of crude extracts and essential oil of *Moringa oleifera* Lam. *Bioresource Technology*, *98*(1), 232–6.

Clarke, J. D., Dashwood, R. H., & Ho, E. (2008, October 8). Multi-targeted prevention of cancer by sulforaphane. *Cancer Letters*, 139,12-16

de Graaf, R. M., Krosse, S., Swolfs, A. E. M., te Brinke, E., Prill, N., Leimu, R.,van Dam, N. M. (2015). Isolation and identification of 4-α-rhamnosyloxy benzyl glucosinolate in *Noccaea caerulescens* showing intraspecific variation. *Phytochemistry*, *110*, 166–171.

Devès, M., & Bourrat, F. (2012). Transcriptional mechanisms of developmental cell cycle arrest: problems and models. *Seminars in Cell & Developmental Biology*, *23*(3), 290–7.

Dhanasekaran, S., Jaganathan, R., Panchanadham, S., & Palanivelu, S. (2012). Induction of Mitochondrion-mediated Apoptosis by Semecarpus anacardium in the BCR–ABL+ 12B1 Leukemia Cell Line: Possible Mechanism of Therapeutic Action In Vivo. *Journal of Experimental & Clinical Medicine*, *4*(1), 30–38.

Dhawan, D., & Gupta, J. (n.d.). 2016.Comparison of different solvents for phytochemical extraction potential from Datura metel plant leaves.. *International Journal of Biological Chemistry 11: 17-22*

Dickinson, S. E., Olson, E. R., Levenson, C., Janda, J., Rusche, J. J., Alberts, D. S., & Bowden, G. T. (2014). A novel chemopreventive mechanism for a traditional medicine: East Indian sandalwood oil induces autophagy and

cell death in proliferating keratinocytes. *Archives of Biochemistry and Biophysics*, *558*, 143–52.

Doppalapudi, R. S., Riccio, E. S., Rausch, L. L., Shimon, J. A, Lee, P. S., Mortelmans, K. E. & Mirsalis, J. C. (2007). Evaluation of chemopreventive agents for genotoxic activity. *Mutation Research*, *629*(2), 148–60.

Dorai, T., & Aggarwal, B. B. (2004). Role of chemopreventive agents in cancer therapy. *Cancer Letters*, (215) 129–140

Dwivedi, A., Pal, M. K., Tripathi, A. K., Yadav, N., Mujtaba, S. F., Pant, M. C., & Manjunatha Prabhu, B. H. (2013). Role of type-II pathway in apoptotic cell death induction by photosensitized CDRI-97/78 under ambient exposure of UV-B. *Toxicology Letters*, *222*(2), 122–31.

Ferreira, P. M. P., Carvalho, A. F. U., Farias, D. F., Cariolano, N. G., Melo, V. M. M., Queiroz, M. G. R., & Machado-Neto, J. G. (2009). Larvicidal activity of the water extract of *Moringa oleifera* seeds against Aedes aegypti and its toxicity upon laboratory animals. *Anais Da Academia Brasileira de Ciências*, *81*, 207–16.

Fimognari, C., Berti, F., Iori, R., Cantelli-Forti, G., & Hrelia, P. (2005). Micronucleus formation and induction of apoptosis by different isothiocyanates and a mixture of isothiocyanates in human lymphocyte cultures. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *582*, 1–10.

Fimognari, C., Turrini, E., Ferruzzi, L., Lenzi, M., & Hrelia, P. (2012). Natural isothiocyanates: genotoxic potential versus chemoprevention. *Mutation Research*, *750*(2), 107–31.

Flora, S. J. S., & Pachauri, V. (2011). Moringa (Moringa oleifera) Seed Extract and the Prevention of Oxidative Stress. *Nuts and Seeds in Health and Disease Prevention*. (pp 775-785) Academic press.

Fox, J. L., & Storey, A. (2015). BMX Negatively Regulates BAK Function, Thereby Increasing Apoptotic Resistance to Chemotherapeutic Drugs. *Cancer Research*, *75*, 1345–1355.

Galuppo, M., Giacoppo, S., De Nicola, G. R., Iori, R., Navarra, M., Lombardo, G. E., & Mazzon, E. (2014). Antiinflammatory activity of glucomoringin isothiocyanate in a mouse model of experimental autoimmune encephalomyelitis. *Fitoterapia*, *95*, 160–74.

Ghosh, N. (2013). Anticancer effect of *Moringa oleifera* leaf extract on human breast cancer cell. Master thesis ,*Jadavpur University*, 1–57.

Giacoppo, S., Galuppo, M., De Nicola, G. R., Iori, R., Bramanti, P., & Mazzon, E. (2015). 4(α-l-Rhamnosyloxy)-benzyl isothiocyanate, a bioactive phytochemical that attenuates secondary damage in an experimental model of spinal cord injury. *Bioorganic & Medicinal Chemistry*, *23*(1), 80–88.

Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). Moringa oleifera: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness*, *5*(2), 49–56.

Guevara, A. P., Vargas, C., Sakurai, H., Fujiwara, Y., Hashimoto, K., Maoka, T., & Nishino, H. (1999). An antitumor promoter from *Moringa oleifera* Lam. *Food and Chemical Toxicology 48*, 345–355 .

Gupta, M., & Gupta, S. (2014). Qualitative and quantitative analysis of phytochemicals and pharmacological value of some dye yielding medicinal plants. *Food Chemistry 105* (2007) 376–382

Gupta, S. (2007). Prostate cancer chemoprevention: current status and future

prospects. *Toxicology and Applied Pharmacology*, *224*(3), 369–76.
Gupta, S. C., Kim, J. H., Prasad, S., & Aggarwal, B. B. (2010). Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. *Cancer and Metastasis Reviews*, *29*, 405–434.
Ha, B., Horie, S., & Chiong, E. (2019). The incidence , mortality , and risk factors of prostate cancer in Asian men. *Prostate International*, *7*, 1–8.
Haas, G. P., & Sakr, W. A. (1997). Epidemiology of prostate cancer. *Ca Cancer J Clin*, *47*(5), 273–287.
Habtemariam, S. (2017). Pharmaceutica Analytica Acta Methodology for Rapid Isolation of Moringin : Potential Anticancer Compound from the Seeds of *Moringa stenopetala*. *Pharmaceutica Analytica Acta*, *8*(8), 105.
Hamza, A. A. (2010). Ameliorative effects of *Moringa oleifera* Lam seed extract on liver fibrosis in rats. *Food and Chemical Toxicology*, *48*(1), 345–355.
Hamza, A. A. (2010). Ameliorative effects of *Moringa oleifera* Lam seed extract on liver fibrosis in rats. *Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association*, *48*(1), 345–55.
Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell*, *144*(5), 646–674.
Hossain, M. A., AL-Raqmi, K. A. S., AL-Mijizy, Z. H., Weli, A. M., & Al-Riyami, Q. (2013). Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. *Asian Pacific Journal of Tropical Biomedicine*, *3*(9), 705–710.
Hseu, Y., Lee, C., Chen, Y., Kumar, K. J. S., Chen, C., Huang, Y., Yang, H. (2014). The anti-tumor activity of Antrodia salmonea in human promyelocytic leukemia (HL-60) cells is mediated via the induction of G 1 cell-cycle arrest and apoptosis in vitro or in vivo. *Journal of Ethnopharmacology*, *153*(2), 499–510.
Issa, R. A. (2010). Identification of glucosinolate profile in Brassica oleracea for quantitative trait locus mapping. *(Unpublished doctoral dissertation*) University of Warwick, UK
Jabeen, R., Mustafa, G., Ul Abdin, Z., Iqbal, M. J., & Jamil, A. (2014). Expression profiling of bioactive genes from Moringa oleifera. *Applied Biochemistry and Biotechnology*, *174*(2), 657–66.
Jan, C., Chen, C., Wang, S., & Kuo, S. (2011). Journal of the Taiwan Institute of Chemical Engineers Phenethyl isothiocyanate induces Ca^{2+} movement and cytotoxicity in PC3 human prostate cancer cells. *Journal of the Taiwan Institute of Chemical Engineers*, *42*(6), 895–901.
Jeeshna, M. V., & Paulsamy, S. (2011). I Phytochemistry and bioinformatics approach for the evaluation of medicinal properties of the herb, exacum bicolor roxb. *International Research Journal of Pharmacy 2 (8), 163-168.*
Jendrossek, V. (2013). Targeting apoptosis pathways by Celecoxib in cancer. *Cancer Letters*, *332*(2), 313–24.
Jia, Y., Ohanyan, A., Lue, Y.-H., Swerdloff, R. S., Liu, P. Y., Cohen, P., & Wang, C. (2015). The effects of humanin and its analogues on male germ cell apoptosis induced by chemotherapeutic drugs. *Apoptosis*, *20*, 551–561.
Johnson, J. J., Bailey, H. H., & Mukhtar, H. (2010). Phytomedicine Green tea polyphenols for prostate cancer chemoprevention : A translational perspective. *Phytomedicine*, *17*(1), 3–13.
Juge, N., Mithen, R. F., & Traka, M. (2007). Molecular basis for

chemoprevention by sulforaphane: a comprehensive review. *Cellular and Molecular Life Sciences : CMLS*, *64*(9), 1105–27.

Kavitha, C., Ramesh, M., Kumaran, S. S., & Lakshmi, S. A. (2012). Toxicity of Moringa oleifera seed extract on some hematological and biochemical profiles in a freshwater fish, Cyprinus carpio. *Experimental and Toxicologic Pathology : Official Journal of the Gesellschaft Für Toxikologische Pathologie*, *64*(7–8), 681–7.

Keum, Y.-S., Jeong, W.-S., & Kong, a N. T. (2004). Chemoprevention by isothiocyanates and their underlying molecular signaling mechanisms. *Mutation Research*, *555*(1–2), 191–202.

Kim, B., & Giardiello, F. M. (2011). Chemoprevention in familial adenomatous polyposis. *Best Practice & Research. Clinical Gastroenterology*, *25*(4–5), 607–22.

Klein, E. a. (2005). Chemoprevention of prostate cancer. *Critical Reviews in Oncology/Hematology*, *54*(1), 1–10.

Kundu, J., Chun, K.-S., Aruoma, O. I., & Kundu, J. K. (2014). Mechanistic perspectives on cancer chemoprevention/chemotherapeutic effects of thymoquinone. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *768*, 22–34.

Lalas, S., & Tsaknis, J. (2002). Characterization of Moringa oleifera Seed Oil Variety “Periyakulam 1.” *Journal of Food Composition and Analysis*, *15*(1), 65–77.

Lee, Y., Jee, Y., Joo, Y., Won, J., Lee, S., & Won, H. (2012). Enhancement of cisplatin cytotoxicity by benzyl isothiocyanate in HL-60 cells. *Food and chemical toxicology*, *50*(7), 2397–2406.

Lettre, D. P., Mishra, G., Singh, P., Verma, R., Kumar, S., & Srivastav, S. (2011). Traditional uses , phytochemistry and pharmacological properties of *Moringa oleifera* plant : An overview. *Der Pharmacia Letter, 3*(2), 141–164.

Lissner, L., Olsson, H., & Gullberg, B. (2019). Measures of birth size in relation to risk of prostate ¨ Diet and Cancer Study , Sweden cancer : the Malmo, *3*(2012), 442–449.

Liu, B., Yang, J., Ma, Y., Yuan, E., & Chen, C. (2010). Antioxidant and angiotensin converting enzyme (ACE) inhibitory activities of ethanol extract and pure flavonoids from *Adinandra nitida* leaves. *Pharmaceutical Biology*, *48*(12), 1432–1438.

Liu, Y., Uemura, H., Ye, D., Lee, J. Y., Chiong, E., Pu, Y., Kooten, & M. Van. "Prostate cancer in Asia : design of a patient registry to inform real-world treatments , outcomes , and quality of life". *Prostate International 1, 1-6* .(2019).

Lockshin, R. a, & Zakeri, Z. (2004). Caspase-independent cell death. *Oncogene*, *23*, 2766–2773.

Lojanapiwat, B., Youl, J., Gang, Z., Kim, C., Chi, N., Hakim, L., Akaza, H. (2018). Report of the third Asian Prostate Cancer study meeting, 4–6.

Luo, J., & Yu, Y. P. (2019). Genetic factors underlying prostate cancer, *5*, 1–26.

Mahmud A, Azizah I, Nor Saleha A, Hashimah Z.A, Asmah W., & Mastulu M. (2016). Malaysian National Cancer Registry Report, *16*.

Mahmood, Z., & Shukla, Y. (2010). Death receptors: targets for cancer therapy. *Experimental Cell Research*, *316*(6), 887–99.

Mahmoud, A. M., Yang, W., & Bosland, M. C. (2014). Soy isoflavones and

prostate cancer: a review of molecular mechanisms. *The Journal of Steroid Biochemistry and Molecular Biology*, *140*, 116–32.

Malabed, R. S., & Noel, M. G. (2013). Characterization of the glucosinolates and isothiocyanates in malunggay (M*oringa oleifera l.*) extracts and determination of their myrosinase activity and anticancer properties, 1–6. Research Congress De La Salle University,Manila, March 7-9, 2013.

Mbikay, M. (2012). Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: A review. *Frontiers in Pharmacology*, *3*, 1–12.

Miñana, B., Rodríguez-Antolín, A., Gómez-Veiga, F., Hernández, C., Suárez, J. F., Fernández-Gómez, J. M.,& Cózar, J. M. (2016). Treatment trends for clinically localized prostate cancer. National population analysis: GESCAP group. *Actas Urológicas Españolas (English Edition)*, *40*(4), 209–216.

Monn, M. F., H, M. P., Tatem, A. J., D, M., Cheng, L., & D, M. (2016). Prevalence and management of prostate cancer among East Asian men : Current trends and future perspectives.*Urologic Oncology: Seminars and Original Investigations 34* (5834) 1–9.

Moon, Y. J., Wang, X., & Morris, M. E. (2006). Dietary flavonoids: effects on xenobiotic and carcinogen metabolism. *Toxicology in Vitro : An International Journal Published in Association with BIBRA*, *20*(2), 187–210.

Morote, J., Maldonado, X., & Morales-Bárrera, R. (2016). Prostate cancer. *Medicina Clínica (English Edition)*, *146*(3), 121–127.

Moshkowitz, M., Shapira, S., & Arber, N. (2011). Chemoprevention for advanced CR neoplasia. *Best Practice & Research. Clinical Gastroenterology*, *25*(4–5), 623–30.

Olden, K., & Vulimiri, S. V. (2014). Laboratory to community: chemoprevention is the answer. *Cancer Prevention Research (Philadelphia, Pa.)*, *7*(7), 648–52.

Pal, S., Bhattacharjee, A., Ali, A., Mandal, N. C., Mandal, S. C., & Pal, M. (2014). Chronic inflammation and cancer: potential chemoprevention through nuclear factor kappa B and p53 mutual antagonism. *Journal of Inflammation (London, England)*, *11*, 23.

Paschka, a G., Butler, R., & Young, C. Y. (1998). Induction of apoptosis in prostate cancer cell lines by the green tea component, (-)-epigallocatechin-3-gallate. *Cancer Letters*, *130*, 1–7.

Peisch, S. F., Van Blarigan, E. L., Chan, J. M., Stampfer, M. J., & Kenfield, S. A. (2017). Prostate cancer progression and mortality: a review of diet and lifestyle factors HHS Public Access. *World J0urnal of Urology*,*35*(6), 867–874.

Pereira Soares, N. D. C., Teodoro, A. J., Oliveira, F. L., Takiya, C. M., Junior, A. P., Nasciutti, L. E., Borojevic, R. (2014). Lycopene induce apoptosis in human prostate cells and alters the expression of Bax and Bcl-2 genes. *Food Science and Technology*, *59*(2), 1290–1297.

Pham, T., Fujino, Y., Kubo, T., Ide, R., Mizoue, T., Ogimoto, I., & Yoshimura, T. (2008). Fish intake and the risk of fatal prostate cancer : findings from a cohort study in Japan. *American Cancer Society Guidelines on Nutrition and Physical Activity for Cancer Prevention*, *12*(5), 609–613.

Pitchakarn, P., Suzuki, S., Ogawa, K., Pompimon, W., Takahashi, S., Asamoto, M., & Shirai, T. (2011). Induction of G1 arrest and apoptosis in androgen-

dependent human prostate cancer by a triterpenoid from *Momordica charantia* leaf. *Cancer Letters*, *306*(2), 142–50.

Premi, M., & Sharma, H. K. (2017). Effect of extraction conditions on the bioactive compounds from *Moringa oleifera* seeds and their identification using LCMS. *Journal of Food Measurement and Characterization*, *11*(1), 213–225.

Qiao, L., & Wong, B. C. Y. (2009). Targeting apoptosis as an approach for gastrointestinal cancer therapy. *Drug Resistance Updates : Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy*, *12*(3), 55–64.

Ragasa, C. Y., Levida, R. M., Don, M.J., & Shen, C.,C. (2012). Cytotoxic isothiocyanates from *Moringa oleifera* Lam seeds. *Philippine Science Letters*, *5*(1), 46–52.

Rajan, T. S., De Nicola, G. R., Iori, R., Rollin, P., Bramanti, P., & Mazzon, E. (2016). Anticancer activity of glucomoringin isothiocyanate in human malignant astrocytoma cells. *Fitoterapia*, *110*, 1–7.

Ramabulana, T., Mavunda, R. D., Steenkamp, P. A., Piater, L. A., Dubery, I. A., Ndhlala, A. R., & Madala, N. E. (2017). Gamma radiation treatment activates glucomoringin synthesis in *Moringa oleifera*. *Brazilian Journal of Pharmacognosy*, *27*(5), 569–575.

Ren, Q., Xing, H., Bao, Z., Su, B., Yang, Q., Yang, Y., & Zhang, Z. (2013). Recent Advances in Separation of Bioactive Natural Products. *Chinese Journal of Chemical Engineering*, *21*(9), 937–952.

Ren, W., Qiao, Z., Wang, H., Zhu, L., & Zhang, L. (2003). Flavonoids: promising anticancer agents. *Medicinal Research Reviews*, *23*(4), 519–34.

Roomi, M. W., Shanker, N., Niedzwiecki, A., & Rath, M. (2015). Induction of Apoptosis in the human prostate cancer cell Line DU-145 by a Novel Micronutrient Formulation. *Open Journal of Apoptosis, 4*, 11-21

Saha, D. (2013). Moringa species (Moringaceae): phytochemistry , cancer chemoprevention potentials with advanced traditional medicinal practice. *Tanzania Journal of Applied Sciences 4*(1), 634–636.

Saldanha, S. N., Kala, R., & Tollefsbol, T. O. (2014). Molecular mechanisms for inhibition of colon cancer cells by combined epigenetic-modulating epigallocatechin gallate and sodium butyrate. *Experimental Cell Research*, *324*(1), 40–53.

Schaefer, E. a M., Stohr, S., Meister, M., Aigner, A., Gudermann, T., & Buech, T. R. H. (2013). Stimulation of the chemosensory TRPA1 cation channel by volatile toxic substances promotes cell survival of small cell lung cancer cells. *Biochemical Pharmacology*, *85*(3), 426–38.

Schmitges, J., Karakiewicz, P. I., Sun, M., Abdollah, F., Budäus, L., Isbarn, H., & Steuber, T. (2012). Predicting the risk of lymph node invasion during radical prostatectomy using the European association of urology guideline nomogram: A validation study. *European Journal of Surgical Oncology (EJSO)*, *38*(7), 624–629.

Shah, S. W., & Aziz, N. A. (2014). Morphology of the lingual apparatus of the Swiftlet, Aerodramus fuciphagus (Aves, Apodiformes, Apodidae). *Journal of Microscopy and Ultrastructure*, *2*(2), 100–103.

Slesser, P., Khan, F., Chau, I., Khan, A Z., Mudan, S., Tekkis, P. P., & Rao, S. (2015). The effect of a primary tumour resection on the progression of synchronous colorectal liver metastases: An exploratory study. *European Journal of Surgical Oncology : The Journal of the European Society of*

Surgical Oncology and the British Association of Surgical Oncology, *41*(4), 484–492.

Small, E. J. (1997). Prostate cancer. *Current Opinion in Oncology*, *9*(3), 277–286.

Sobue, S., Mizutani, N., Aoyama, Y., Kawamoto, Y., Suzuki, M., Nozawa, Y., & Murate, T. (2016). Mechanism of paclitaxel resistance in a human prostate cancer cell line, PC3-PR, and its sensitization by cabazitaxel. *Biochemical and Biophysical Research Communications*, *479*(4), 808–813.

Song, L., Morrison, J. J., Botting, N. P., & Thornalley, P. J. (2005). Analysis of glucosinolates, isothiocyanates, and amine degradation products in vegetable extracts and blood plasma by LC-MS/MS. *Analytical Biochemistry*, *347*, 234–243.

Song, X., Zhang, Y., Wang, X., Zhang, W., Wang, Z., Zhang, F., & Gu, J. (2017). Casticin induces apoptosis and G0/G1 cell cycle arrest in gallbladder cancer cells. *Cancer Cell International*, *17*(1), 9.

Sothilingam, S., Sundram, M., Malek, R., & Sahabuddin, R. M. (2010). Prostate cancer screening perspective, Malaysia. *Urologic Oncology*, *28*(6), 670–2.

Sreelatha, S., Jeyachitra, A., & Padma, P. R. (2011). Antiproliferation and induction of apoptosis by Moringa oleifera leaf extract on human cancer cells. *Food and Chemical Toxicology*, *49*(6), 1270–1275.

Srivastava, S. K., & Singh, S. V. (2004). Cell cycle arrest, apoptosis induction and inhibition of nuclear factor kappa B activation in anti-proliferative activity of benzyl isothiocyanate against human pancreatic cancer cells. *Carcinogenesis*, *25*(9), 1701–9.

Stearns, M. E., & Wang, M. (2011). Synergistic Effects of the Green Tea Extract Epigallocatechin-3-gallate and Taxane in Eradication of Malignant Human Prostate Tumors. *Translational Oncology*, *4*(3), 147–156.

Stuart, E. C., Scandlyn, M. J., & Rosengren, R. J. (2006). Role of epigallocatechin gallate (EGCG) in the treatment of breast and prostate cancer. *Life Sciences*, *79*, 2329–2336.

Suphachai, C. (2014). Antioxidant and anticancer activities of Moringa oleifera leaves. *Journal of Medicinal Plants Research*, *8*(7), 318–325.

Surh, Y.J. (2003). Cancer chemoprevention with dietary phytochemicals. *Nature Reviews. Cancer*, *3*(10), 768–80.

Tai, S., Sun, Y., Squires, J. M., Zhang, H., Oh, W. K., Liang, C. Z., & Huang, J. (2011). PC3 is a cell line characteristic of prostatic small cell carcinoma. *Prostate*, *71*(15), 1668–1679.

Thapa, D., & Ghosh, R. (2012). Antioxidants for prostate cancer chemoprevention: challenges and opportunities. *Biochemical Pharmacology*, *83*(10), 1319–30.

Tiloke, C., Phulukdaree, A., & Chuturgoon, A. A. (2016). The antiproliferative effect of *Moringa oleifera* crude aqueous leaf extract on human esophageal cancer cells. *Journal of Medicinal Food*, *19*(4), 398–403.

Tiloke, C., Phulukdaree, A., & Chuturgoon, A. A. (2013). The antiproliferative effect of *Moringa oleifera* crude aqueous leaf extract on cancerous human alveolar epithelial cells. *BMC Complementary and Alternative Medicine*, *13*(1), 226.

Vaish, V., & Sanyal, S. N. (2012). Role of Sulindac and Celecoxib in chemoprevention of colorectal cancer via intrinsic pathway of apoptosis :

Exploring NHE-1 , intracellular calcium homeostasis and Calpain 9. *Biomedicine et Pharmacotherapy*, *66*(2), 116–130.

Vasanth, K., Ilango, K., MohanKumar, R., Agrawal, A., & Dubey, G. P. (2014). Anticancer activity of *Moringa oleifera* mediated silver nanoparticles on human cervical carcinoma cells by apoptosis induction. *Colloids and Surfaces. B, Biointerfaces*, *117*, 354–9.

Viollet, B., Guigas, B., Sanz Garcia, N., Leclerc, J., Foretz, M., & Andreelli, F. (2011). Cellular and molecular mechanisms of metformin: an overview. *Clinical Science*, *122*(6), 253–270.

Wen, S., Niu, Y., Lee, S. O., & Chang, C. (2014). Androgen receptor (AR) positive vs negative roles in prostate cancer cell deaths including apoptosis, anoikis, entosis, necrosis and autophagic cell death. *Cancer Treatment Reviews*, *40*(1), 31–40.

Wu, X., Patterson, S., & Hawk, E. (2011). Chemoprevention--history and general principles. *Best Practice & Research. Clinical Gastroenterology*, *25*(4–5), 445–59.

Xu, H., Yu, X., Qu, S., & Sui, D. (2013). Juglone, isolated from Juglans mandshurica Maxim, induces apoptosis via down-regulation of AR expression in human prostate cancer LNCaP cells. *Bioorganic and Medicinal Chemistry Letters*, *23*(12), 3631–3634.

Xu, M., & Kim, Y. S. (2014). Antitumor activity of glycyrol via induction of cell cycle arrest, apoptosis and defective autophagy. *Food and Chemical Toxicology*, 74, 311-9

Zade, V. S., Dabhadkar, D. K., Thakare, V. G., & Pare, S. R. (2013). Effect of aqueous extract of *Moringa oleifera* seed on sexual activity of male albino rats. *Biological Forum – An International Journal*, *5*(1), 129–140.

Zagouri, F., Peroukidis, S., Tzannis, K., Kouloulias, V., & Bamias, A. (2015). Current clinical practice guidelines on chemotherapy and radiotherapy for the treatment of non-metastatic muscle-invasive urothelial cancer: A systematic review and critical evaluation by the Hellenic Genito-Urinary Cancer Group (HGUCG). *Critical Reviews in Oncology/Hematology*, *93*(1), 36–49.

Zhao, L., Su, J., Li, L., Chen, J., Hu, S., Zhang, X., & Chen, T. (2014). Mechanistic elucidation of apoptosis and cell cycle arrest induced by 5-hydroxymethylfurfural, the important role of ROS-mediated signaling pathways. *Food Research International*, *66*, 186–196.