REVIEW

Moringa oleifera Lam: Targeting Chemoprevention

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Abstract

Moringa oleifera **Lam, family Moringaceae, is a perennial plant which is called various names, but is locally known in Malaysia as ''murungai" or ''kelor". Glucomoringin, a glucosinolate with from *M. oleifera* is a major secondary metabolite compound. The seeds and leaves of the plant are reported to have the highest amount of glucosinolates. *M. oleifera* is well known for its many uses health and benefits. It is claimed to have nutritional, medicinal and chemopreventive potentials. Chemopreventive effects of *M. oleifera* are expected due to the existence of glucosinolate which it is reported to have the ability to induce apoptosis in anticancer studies. Furthermore, chemopreventive value of *M. oleifera* has been demonstrated in studies utilizing its leaf extract to inhibit the growth of human cancer cell lines. This review highlights the advantages of *M. oleifera* targeting chemoprevention where glucosinolates could help to slow the process of carcinogenesis through several molecular targets. It is also includes inhibition of carcinogen activation and induction of carcinogen detoxification, anti-inflammatory, anti-tumor cell proliferation, induction of apoptosis and inhibition of tumor angiogenesis. Finally, for synergistic effects of *M. oleifera* with other drugs and safety, essential for chemoprevention, it is important that it safe to be consumed by human body and works well. Although there there were promising evident about *M. oleifera* in chemoprevention extensive research need to be done due to the expected rise of cancer in coming years and to gain more information about the mechanisms involved in *M. oleifera* influence, which could be a good source to inhibit several major mechanisms involved in cancer development.**

Keywords: *Moringa oleifera* - glucosinolate - glucomoringin - chemopreventive

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Origin and Distribution of *M. Oleifera*

Moringa oleifera Lam. (*M. oleifera*) comes from the family Moringaceae in phylogeny tree. It is widely distributed and known native in India, Afghanistan, Bangladesh, and Pakistan (Amaglo et al., 2010). *M. oleifera* is also called with various names such as horseradish tree, drumstick tree and locally named 'murungai' or 'kelor'. The plant is known to survive the arid environment which makes it easily grown around the world such as in West, East, South Africa, Tropical Asia and Latin America. The plant is edible and consumed by most of the people in Asia including Thailand, Malaysia and India (Ghosh, 2013) *M. oleifera* can be described as a perennial plant, has spirally arranged leaves, whitish flower and low quality of timber (Figure 1) (Ghosh, 2013). Scientific classification (Figure 2) shows that the plant is in order of Brassicales and is one of the cruciferous plants (Ganesan et al., 2014). The characteristics of the plant in order of Brassicales is it contains glucosinolates as their exclusive compound (Gueyrard et al., 2000; Rollin and Tatibouet, 2011; Galuppo et al., 2013; Forster et al., 2015). Nowadays, Malaysians grow the plant to get constant source for food or use in traditional medicine. There are companies that grow the plant in large production to be sold as ingredient for medicinal purposes or research studies. The awareness of most people to consume high nutritional food from natural sources caused high demand of the raw *M. oleifera* in the market.

Uses of *M. oleifera*

M. oleifera is usually used in traditional medicine as ingredients because of its pharmacological properties. Some of the claimed properties, which had been proven in research were anti-bacteria and anti-tumor (Anwar et al., 2007). In pharmaceuticals industry, it had been used with some other ingredients to produce medicine. The plant had also been used to purify water, especially the seed (Tahir et al., 2010). The seed had been used as coagulant, to treat water turbidity of any impurities (Santos et al., 2005; Katayon et al., 2006; Gupta et al., 2010). It is

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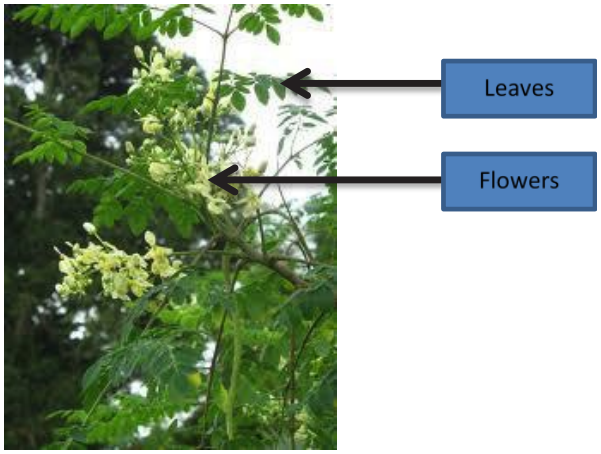


Figure 1. Leaves and flowers of *M. oleifera* (Source: Ghosh, 2013)

Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Brassicales

Family: Moringaceae

Genus: *Moringa*

Species: *Moringa oleifera*

Binomial name: *Moringa oleifera* Lam.

Figure 2. Taxonomic Classification of *M. oleifera* (Source: Garima et al., 2011)

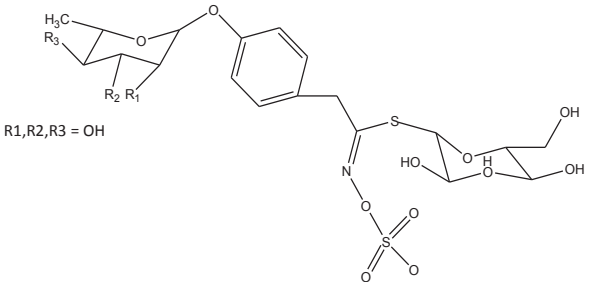


Figure 3. Structure of a Glucosinolate Molecule from *M. oleifera*. (Source: Förster et al., 2015)

also used to eliminate microbial in flocculated water and able to remove metals in liquid solution (Popoola and Obembe, 2013). In biodiesel field, the oil from the seed had been developed for biofuel which is environmental friendly (Rashid et al., 2008). Therefore, there are many uses of *M. oleifera* including ornamental plant, pesticide, medicine, cleaning agent, fencing, and biogas, used in haircare products and as food for chicken (Anwar et al., 2007; Ghosh 2013).

Phytochemical composition of *M. oleifera*

Several phytochemicals present in *Moringa oleifera* Lam. which distributed in its leaf, branch, seed, pod and root. Several major phytochemicals of *M. oleifera* are glucosinolates, phenolics, flavonoids, crude fats, fatty

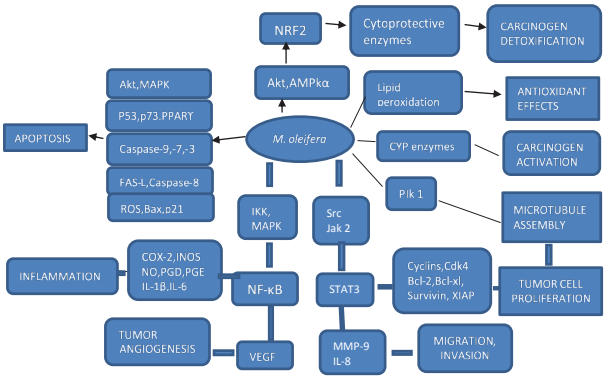


Figure 4. Proposed Molecular Targets of *M. oleifera* as a Chemopreventive Agent (Source: Kundu et al. 2014)

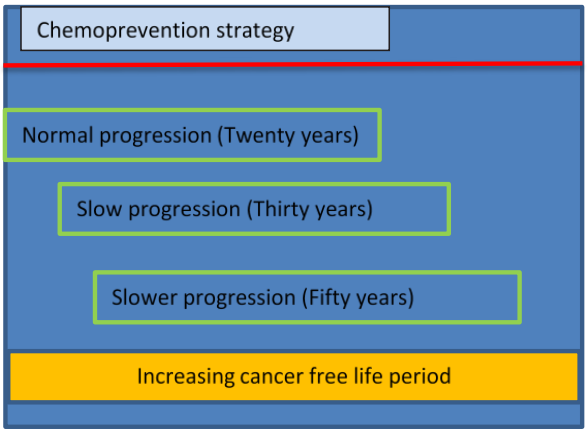


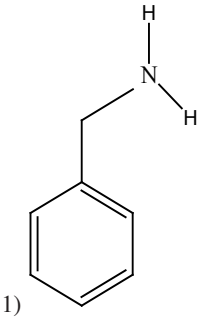
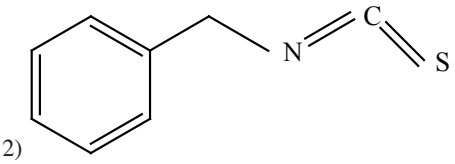
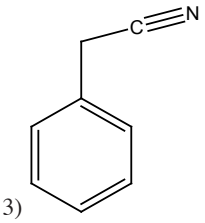
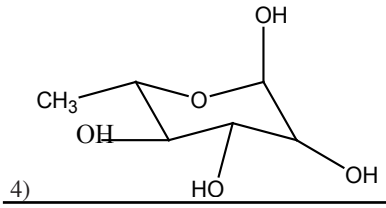
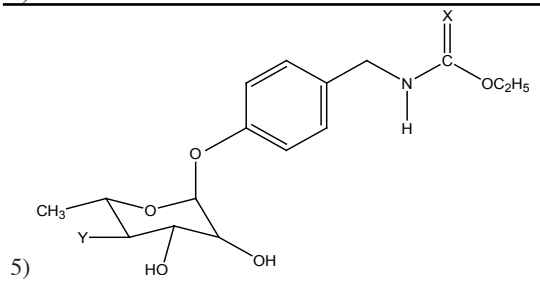
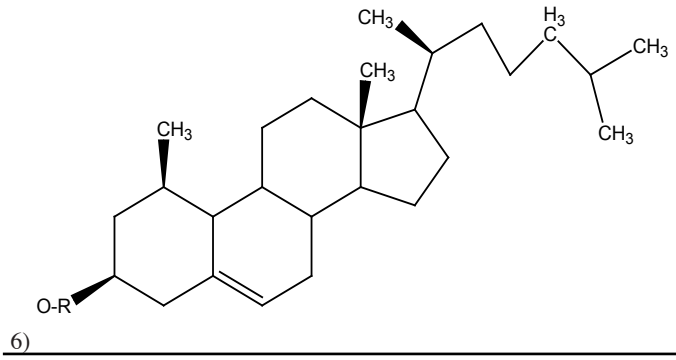
Figure 5. Slowing the Process of Carcinogenesis to Increase Cancer Free Life Period

Table 1. Amino Acid Content in Fresh and Dried Leaves of M. oleifera per Gram (g) Edible Portion (Source: Shruti et al., 2011)

Amino acid	Fresh leaf (g)	Dried leaf (g)
Arginine	0.041	0.133
Histidine	0.149	0.613
Isoleucine	0.299	0.825
Leucine	0.492	0.195
Lysine	0.342	0.133
Methionine	0.117	0.35
Phenylalanine	0.31	0.139
Threonine	0.117	0.119
Tryptophan	0.107	0.425
Valine	0.374	0.106

acid, major nutrient, mineral and total protein (Flora and Pachauri, 2011). In addition, glucosinolates are the secondary metabolites that indicates the characteristic of Brassicales plant including *M. oleifera* where it only can be found in the order of Brassicales (Garima et al., 2011). Glucosinolates are most abundant in most part of *M. oleifera* plant except the root (Ghosh, 2013). *M. oleifera* contains high amount of aromatic glucosinolates such as p-hydroxybenzyl glucosinolates (sinalbin), 2-phnylethyl glucosinolates (gluconasturtiin), benzyl glucosinolates (glucotropaeolin) (Forster et al., 2015). In addition, benzyl glucosinolates is the dominant compound presented in the

Table 2. Structure of Major Phytochemicals from seeds of *M. oleifera* (Source: Flora and Pachauri, 2011)

 1)	1) Alkaloid: moringin
 2)	2) Flavonoids Glycosides: Benzyl isothiocyanate and its derivatives
 3)	3) (α-L-rhamnosyloxy), phenylacetonitrile, 4-hydroxyl phenyl-acetonitrile, and 4-hydroxyphenyl-acetamide
 4)	4) 4-(α-L-rhamnosyloxy), 4-(α-L-rhamnosyloxy), phenylacetonitrile (β-carotene), sterols and lecithin
 5)	5) O-ethyl-4-(α-L-rhamnosyloxy), benzylcarbamide with seven derivatives
 6)	6) Nutrients: Vitamin B1, B6, riboflavin, folic acid, nicotinic acid, vitamin C and E

leaf of *M. oleifera* (Forster et al., 2015). Seed and leaf of *M. oleifera* have the highest glucosinolates content compared to other parts of plant (Ghosh, 2013). The leaf usually has high flavonoid value whereas the seed has high crude protein value (Ghosh, 2013). In many reported journals, the leaves extract are the most studied for various uses (Kasolo et al., 2010; Asare et al., 2012; Lambole and Kumar, 2012; Moyo et al., 2012; Ratshilivha et al., 2014). It was claimed that the dried leaf extract showed higher amino acid, vitamin and minerals content than the fresh leaf extracts itself (Ghosh, 2013). Therefore, it is concluded that the dried extract of the plant will give higher yield of purified compound. Table 1 shows amino acid content in fresh and dried leaves of *M. oleifera* in 1 g of edible portion.

Phytochemicals of *M. oleifera* were also reported to have bioactivities such as anti-bacterial, anti-fungus and anti-tumor (Kasolo et al., 2010). Figure 3 shows structure of glucosinolates molecule from *M. oleifera* plant. The functional groups presented in the compound usually

Table 3. Reports on Cancer Chemoprevention Through ion Vitro Studies of *M. oleifera*

Study	Remarks	Reference
Anti-proliferation and induction of apoptosis by Moringa oleifera leaf extract on human cancer cells	Extracts induced apoptosis in human tumor cell line	Sreelatha et al., (2011)
Ethanolic extract of Moringa oleifera increased cytotoxic effect of doxorubicin on HeLa cancer cells	Combination of extract and doxorubicin increased doxorubicin effect through apoptotic induction	Hermawan et al., (2012)
Antioxidant and anticancer activities of Moringa oleifera leaves	Dichloromethane extract showed high antioxidant and potent anti-cancer proliferation	Charoensin (2014)
Moringa species (Moringaceae): phytochemistry, cancer chemoprevention potentials with advanced traditional medicinal practice	Skin tumor prevention and exhibit good hepatoprotective	Dibyajyoti (2013)
Anticancer effect of Moringa oleifera leaf extract on human breast cancer cell	Anti-proliferative effect on 2 breast cancer cell lines, MDA MB 231 and MCF 7, showed a dose and time dependent effect	Ghosh (2013)

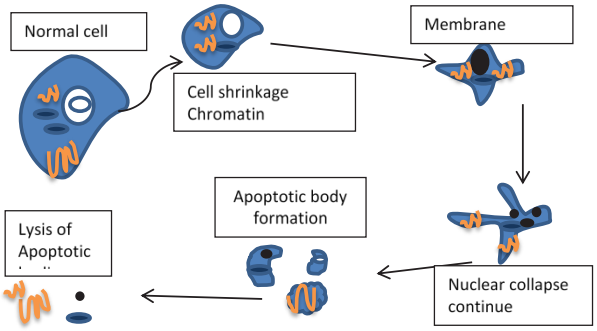


Figure 6. Apoptosis process. (Source: Tanaka, 2013)

Figure 6. Apoptosis Process (Source: Howstuffworks. com, 2010)

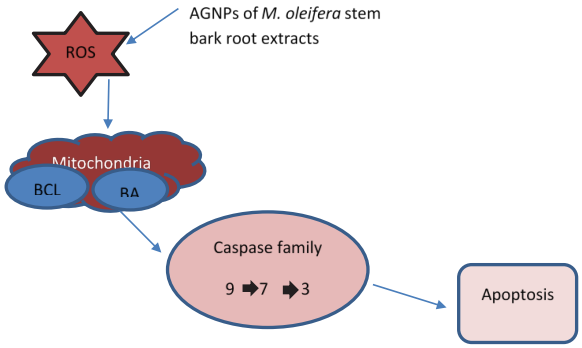


Figure 7. Example of Main Signaling Pathway in Apoptosis Induction of Hela Cells through Ros-Mediated Pathway of *M. Oleifera* Stem Bark Extracts Mediated Silver Nanoparticles (AGNPs)

responsible for the bioactivities triggered.

Brunelli et al. (2010) reported that glucosinolates were found higher in the seeds by 8 % compared to other plant parts. Glucosinolates were reported to induce apoptosis in tumour cell and the major studies had been done on leaf extracts (Forster et al., 2015). While studies on the anti-cancer of the seed extract is very scarce, thus it is important to carry out anti-cancer studies of the seed due to the abundance of glucosinolates presented in the seeds.

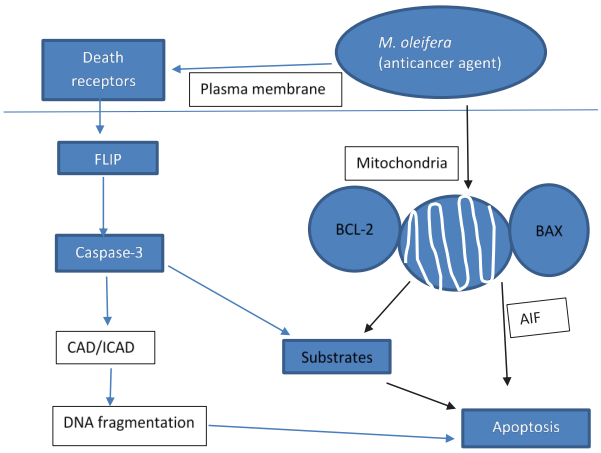


Figure 8. Simulation of Extrinsic and Intrinsic Pathways Activated by *M. oleifera* or Anticancer Agents

Table 2 shows major phytochemicals listed and reported from seeds of *M. oleifera*.

Nutritional and medicinal value of *M. oleifera*

M. oleifera is recognized as a nutritious plant which can be consumed to fight famine due to its highly nutritious content (Trees for life organization, 2005). It has been reported that in 100 gram of dry *M. oleifera* leaf contained 12 times vitamin C than oranges, 10 times vitamin A than carrots, 9 times protein than yoghurt, 15 times potassium than banana, 17 times calcium than milk and 25 times iron than spinach (Tahir et al., 2010). This nutritional content was also responsible in many important function in biochemical process of human and animals (Sauberlich,1984; Senba and Nusemblatt, 2002). The animal and human cannot produce certain types of important nutrition by themselves that are essential in the natural function, so it must be consumed from the outside which this plant can be one of the best nutrition sources (Wu, 2010). In certain countries such as India, Pakistan, Philippines, Hawaii and many parts of Africa, the immature pods, leaves and flowers of the plant had also consumed as a source of vegetable (D'souza and Kulkarni, 1993; Anwar and Bhanger, 2003; Anwar et al., 2005).

M. oleifera extract had been utilized as an ingredient in many Indian traditional (Ghazali and Mohammed, 2011). The plant is said to have various beneficial effects such as anti-inflammatory, anti-fibrotic, anti-microbial, anti-oxidant, anti-hyperglycemic, anti-tumor and anti-cancer (Abdull et al., 2014). It is well known as a multipurpose crop because of the various properties from different part of the plant that contributed to the various beneficial effects (Amaglo et al., 2010). The boiled *M. oleifera* pod extract had exhibited anti-clastogenic activity when 1.5 %, 3.0 % and 6.0 % of the extracts were introduced to mouse by *in vivo* erythrocyte micronucleus assay (Chadamas et al., 2010). It was reported that the seed contains major phytochemicals with reported medicinal value (Fuglie et al., 2001; Chuang et al., 2007; Ghazali et al., 2011). In previous pharmacological studies, glucomoringin was reported to be able to relax bronchioles and exert liver protective effects (Mahajan and Mehta, 2010). The flavonoids and glycosides possess anti-oxidant, anti-inflammatory, anti-microbial, anti-cancer and anti-hypertensive activities (Brunelli et al., 2010; Sudha et al., 2010; Galuppo et al., 2014). The (α-L-rhamnosyloxy), phenylacetonitrile, 4-hydroxyl phenyl-acetonitrile, and 4-hydroxyphenyl-acetamide have mutagenic property (Galuppo et al., 2014). 4-(α-L-rhamnosyloxy), 4-(α-L-rhamnosyloxy), phenylacetonitrile (β-carotene), sterols and lecithin have anti-microbial and anti-biotic activities (Ghazali et al., 2011; Galuppo et al., 2014). O-ethyl-4-(α-L-rhamnosyloxy), benzylcarbamide with seven derivatives have antitumor and antihypertensive activities (Chumark et al., 2008). The last components are the nutrients which can provide nutrient and has anti-oxidant property (Garima et al., 2011). *M. oleifera* had also been used to produce commercial medicine and cosmetic products in India such as Rumalaya and Septilin (Himalaya Drug Company), Orthoherb (Walter Bushnell Ltd), Kupid Fort (Pharma Products) and Livospin (Herbals APS) (Mehta et al., 2003). Therefore, the root, leaf, stem bark, gum, flower and seed had been reported to have various medicinal uses (Anwar et al., 2007).

Chemopreventive value and targeting chemoprevention of *M. oleifera*

According to Dr. Michael Sporn (1976), who first defined the word chemoprevention, chemoprevention is "the use of specific agents to reverse, or prevent the carcinogenic process to invasive cancer". A compound that has the ability to induce apoptosis of cancer cell is called chemopreventive agent. Chemopreventive effect of the compound makes the plant as an alternative for anti-cancer. Chemoprevention studies had been an important research in order to combat cancer worldwide. The statistic death caused by cancer was increasing every year, which change of diet and lifestyle of people nowadays become the main influences (Anand et al., 2008). Various types of plant are reported to have phytochemicals that are able to slow down or fight cancer. *Moringa oleifera* possessed glucosinolates, an exclusive compound which is able to induce apoptosis. In fact, the hydrolysis product of glucomoringin (GMG) with, called 4(α-L-rhamnosyloxy)-benzyl isothiocyanate (GMG-ITC) is working effectively better than the non-hydrolysis in inducing apoptosis. There are some studies showed that the crude extract of *M. oleifera* has the ability to inhibit carcinogenesis in cancer cells *in vitro* and *in vivo* (Foti Cuzzola et al., 2013; Galuppo et al., 2013). Vasanth et al. (2014), had reported that silver nanoparticles of *M. oleifera* extract, was discovered to induce apoptosis in human cervical carcinoma cells (HeLa). Furthermore, it was also found that *M. oleifera* pod had exhibited colitis-related colon carcinogenesis inhibition in mouse model induced by genotoxic colon carcinogen, azoxymethane and dextran sodium sulphate (Budda et al., 2011). In the strategy for *M. oleifera* to be a potential chemopreventive agent, the compound should involve in pathways or mechanisms in activating or inhibiting carcinogen detoxification, anti-oxidant effects, tumor cell proliferation, apoptosis, inflammation, tumor angiogenesis, migration, invasion and synergistic effect (Figure 4) (Neergheen et al., 2010; Boghossian and Hawash, 2012). Table 3 shows some reports regarding *in vitro* studies of *M. oleifera* for potential chemopreventive agent.

Cancer chemoprevention: evidence potential from *in vitro* studies

Mechanism of chemoprevention

Glucosinolates showed an effect of inhibiting cancer in different chemoprevention strategy (Wang et al., 2012). In general, the mechanisms that involved in hallmark of cancer are apoptosis inhibition, carcinogen activation and detoxification, tumor proliferation, enzyme modulation and tumor angiogenesis (Kundu et al., 2014). Cancer therapeutic agents will exhibit characteristics to block the mechanisms that involved in inducing cancer processes (Neerghen et al., 2010). Many studies targeting on apoptosis induction in order to determine the chemopreventive effect (Wang et al., 2012).This is because most pathways involved in apoptosis induction are recognized and been described in the previous studies (Kang et al., 1998; Lie-weber et al., 2010). Therefore, *M. oleifera* is expected to involve in several mechanisms of molecular target of cancer prevention as shown in figure 4. In addition, *M. oleifera* extracts were reported to involve in various anti-cancer activities such as apoptosis induction, tumor cell proliferation and anti-inflammatory (Guevara et al., 1999; Verma et al., 2009; Sreelatha et al., 2011).

Inhibition of carcinogen activation and induction of carcinogen detoxification

Carcinogenesis involved various biochemical process which it can be caused by a defect in apoptosis pathways and various xenobiotic-metabolizing enzymes. CYP or P450 is one of the xenobiotic-metabolizing enzyme that activates carcinogen process (Shumada, 2006). If expression of Cytochrome P (CYP) enzyme is inhibited, carcinogenesis will not occur. According to Bharali and colleague (2003), hydro-alcoholic extract of *M.*

oleifera was found to inhibit chemical carcinogenesis of hepatic carcinogen metabolizing enzymes, elucidating that it balanced the xenobiotic metabolism towards detoxification, thus avoiding carcinogenesis. It was also reported to modulate both phase I and phase II system enzymes which are CYP (activate carcinogenesis) and glutathione-S- transferase (GST) (detoxify carcinogenesis) in swiss albino mouse (Banchini and Vanaio, 2001; Rupjyoti Bharali et al., 2003). Carcinogenesis of cells can be caused by various physical, chemical and biological factors (Baba and Catoi, 2007) The physical factors are such as UV rays or radiation, chemical factors usually involved toxic substances and biological factors are natural pollutants or compounds presented in human and animal food (Baba and Catoi, 2007). Preventing carcinogen in the first stage of cancer progression can avoid cancer to occur through targeting enzyme modulation (Banchini and Vanaion, 2001). Hydroethanolic extract of *Moringa oleifera* pod was observed to influence modulation of hepatic xenobiotic (phase I) drug metabolizing enzyme in induced hepatocellular damage in male mice (Sharma and Paliwal, 2012). Therefore, it indicated that the plant exhibited hepatoprotective activities through cytoprotective enzymes where the bioactive compounds presented were responsible for the action.

Alternatively, the opposite of carcinogenesis is carcinogen detoxification in which it can be activated through NRF2 (nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2) signalling pathway (Itoh et al., 1997). It could be a rational approach in preventing cancer by plant based product which contains phytochemicals that usually involved in chemoprevention (Jeong et al., 2011; Wang et al., 2012; Leonarduzzi et al., 2012). Plant compounds induced cytoprotective enzymes pathways (Figure 4) including phase II and anti-oxidative enzymes (through NRF2 pathway) to detoxify and execute dangerous intermediates to form in preventing carcinogenesis (Yang et al., 2001). Isothiocyanates from cruciferous vegetables were reported to induce phase II enzyme through Nrf2 that exhibited tumor inhibitory activity (Zhang et al., 2004). Cytoprotective enzymes which induced carcinogen detoxification were reported to be regulated by antioxidant responsive element (ARE) (Lee and Surh, 2005). When chemopreventive agent is activated, the antioxidant responsive elements (ARE) will be mediated through Nrf2 (Lee and Surh, 2005). So, it is concluded that chemoprevention through the NFR2-ARE pathway is one of the effective strategy to combat cancer activation. Studies suggested that NRF2 responses to inducers through two major mechanisms (Kalaany and Sabatini, 2008). First is down regulation of Nrf2 ubiquitination which disturb the Keap1–Cul3 and Keap1–Nrf2 complexes through modification of cysteine thiols of Keap1, phosphorylation of Nrf2, or both. The second mechanism involves alteration of the nuclear import or export of Nrf2 (Dinkova-Kostova, 2002). Various ARE inducers are usually natural plant compound such as epigallocatechin (EGCG), resveratrol, curcumin, and sulphur – containing compounds (Kou et al., 2013). Kempferol, phenolic compounds, quercetin and isothiocyanates were identified to target NRF2-Keap1-ARE elements in chemoprevention strategy (Zhao et al., 2010). Sulphoraphane, is one of the isothiocyanates was reported to be responsible in increment of phase II enzymes expression at mRNA, protein and activity levels in cell lines. In human prostate cancer cells lines, when sulphoraphane was introduced, it helped to increase the cancer protective genes expression, quinone oxidoreductase 1 (NQO1) and glutathione S-transferase A1 (GSTA1), as well as the augmented activities of microsomal GSTA1 and NQO1 (Zhang et al., 1994). Therefore, it is important to study the potential chemopreventive activity of *M. oleifera* through modulation expression of a well-known cancer-activating gene, Cytochrome P450 1a1 (Cyp1a1), and cancer-protective genes as it will help in the strategy order to slow down the carcinogenesis process in chemoprevention strategy as shown in Figure 5.

Chemoprevention strategy as shown in figure 5 is a plan to increase cancer free life period through various research and studies. Normal progression of cancer is expected to take several periods to become chronic but when avenues of cancer studies and research had been done, the progression of cancer can be slowed down stage by stage until solutions to treat the cancer is finally found (Mukhtar, 2012).

Anti-inflammatory of *M. oleifera*

Induction of anti-inflammatory mediators by phytochemicals are important to avoid the trigger of inflammation by cell (Mueller et al., 2010). This is because inflammation can leads to carcinogenesis through activation of inflammatory mediators such as prostaglandins, cytokines, chemokines and nitric oxides (Kundu et al., 2014). Activation of MAP-kinase family also will activate NF-κB signalling which will induce inflammatory (Galuppo et al., 2014). Therefore, the plant compounds used for treatment must have the ability to inhibit the mediators and signals. Previous study stated that *M. oleifera* leaf extract was able to give anti-inflammatory effects in male albino rats against carrageenan induced hind paw oedema, and it seeds extracts was reported to reduce weight of distal colon which is a marker for inflammation (Rao et al., 1999; Minaiyan et al., 2014) In the studies of experimental autoimmune encephalomyelitis (EAEC) by mouse model, isothiocyanate (4(α-L-rhamnosyloxy)-benzyl isothiocyanate) (GMG-ITC)) reduced cytoplasmic level of protein when compared to control EAEC mouse (Galuppo et al., 2014). The author also stated that the ciplastin protein was reduced after treatment, it is indicated that the compound exhibited anti-inflammatory effect towards the cell, thus provide protective effects towards central nervous system (CNS) tissue. Targeting molecular target to inhibit cyclooxigenase (COX) and nitrogen oxidase (NO) production is a good way in order for inhibitors to exhibit anti-inflammatory effects (Surh and Kundu, 2005; Romagnolo et al., 2010).Various phytochemicals such as polyphenols were reported to have the ability to inhibit COX and NO production in many cancer studies (Wang et al., 2012). In a study of COX-2 as molecular target

to combat colon cancer, nonsteroidal anti-inflammatory drug (NSAIDs) was used to inhibit COX enzyme that was able to exhibit chemopreventive effect in colon cancer (Kaur et al., 2012). When *M. oleifera* compounds or GMG from *M. oleifera* specifically inhibited production of cyclooxygenase and nitrogen oxigenase enzyme with minimal cytotoxicity, thus it can provide sources for chemopreventive agent for various cancer incidences. Various development of tumor can be prevented from the effects of inhibiting COX expression (Soslow et al., 2000). Crude extracts of Saeda fruticosa (S. fruticosa) was found in a study that able to inhibit NO expression in lipopolysaccharides (LPS)-stimulated RAW 264.7 macrophages by 66 % in 0.16g/mL concentration (Oueslati et al., 2012). The authors claimed that they measured the anti-inflammatory activity of S. fruticosa by nitrite quantification.

Anti-tumor cell proliferation of *M. oleifera*

The formation of tumor involved multistage process that happens in series of events and often take a long time (Gupta et al., 2010). The sign of tumor cell proliferation is when P1K1 in biochemical basis of cancer is activated. Thus, by inhibiting the mechanism, it will avoid activation of cell proliferation as well as inhibiting cancer development to occur. It was reported that both methanol and dichloromethane *M. oleifera* leaves extract were able to inhibit cell proliferation of HepG2, Caco-2, MCF-7 and human fibroblast cells (Supachai, 2014). Tumor cell proliferation can occur through cell cycle arrest or quinone oxidoreductase 1 (QR) gene expression (Hanahan and Weiberg, 2000; Lee et al., 2015). In the studies of human tumor cell line, *M. oleifera* leaves extracts were able to reduce cell proliferation by 15 % (Sreelatha et al., 2011).

Cell proliferation occurred in most sequence of carcinoma adenoma such as early and late adenoma. Activation of microtubule assembly will cause the tumor cell proliferation in molecular mechanism to occur. Therefore, if *M. oleifera* phytochemicals are able to inhibit protein signalling that caused the microtubule assembly through cell cycle arrest, thus it can inhibit cell proliferation. Glucomoringin derived-isothiocyanates (GMG-ITC) of *M. oleifera* was also reported to reduce tumor growth in Swiss Ncr nu/nu mice bearing A2780 ovarian cancer (Brunelli et al., 2010). It was suggested that GMG-ITC slowed down the progress of cells through all phases of cell cycle. In order for the compound to exert its effect, it is important for it to show the ability to induce drug metabolizing enzyme such as the phase-II drug metabolizing enzyme and Glutathione-S-Transferase (Li et al., 2009). Benzyl isothiocyanate (BITC) from cruciferous plant was also reported to induce cell cycle arrest at G2/M phase, thus inhibit cell proliferation (Srivastava and Singh, 2004). It indicates that isothiocyanate may able to suppress protein and pathway that involved in tumor cell proliferation such as Cdk4, cyclins, Bcl-2 and Bcl-x.

Induction of apoptosis by *M. oleifera*

Apoptosis is a programme cell's death which plays an important role in normal physiological functions (Levine et al., 2001; Wang et al., 2012). When tumor cells evade from cell death machinery, thus the cell will be transformed into cancer cell (Susan and Brad, 2005). Studies reported that the phytochemical compounds of *M. oleifera* were able to induce apoptosis in cancer cell (Sreelatha et al., 2011; Sharma et al., 2012; Vijay and Kumar, 2012; Waterman et al., 2014).

Apoptosis process as shown in Figure 6 is initiated in two pathways which are intrinsic and extrinsic pathway where the pathways occur with various different signallings and also correlated (Tanaka, 2013). *M. oleifera* leaf extract was reported to cause membrane blebbing and apoptotic bodies to occur in human tumor cell line (KB) when the extract was introduced to the cell line, thus induced apoptosis (Sreelatha et al., 2001). Morphological changes such as membrane blebbing and formation of apoptotic bodies are also one of the morphological alteration of apoptosis (Machuey et al., 2004). The author also stated that *M. oleifera* showed an anti-proliferative effect by morphology changes, causing loss of cell viability, and internucleosomal DNA fragmentation in KB cells due to chemical composition presented in the leaf extracts (Sreelatha et al., 2011). Various signalling and mechanisms involved in apoptosis induction which are also activated by caspase signalling. Extrinsic pathway can be called as caspase-dependent extrinsic apoptosis while intrinsic pathway is also known as caspase-independent intrinsic apoptosis (Wen et al., 2014). In the cytotoxicity study of *M. oleifera* plant extract using brine shrimp (Artemia salina Leach) lethality model, it was found that the extract gave positive result for anti-cancer agent (Ali and Musa, 2012). Brine shrimp lethality test had long been used as preliminary study to determine toxicity of compounds toward the brine shrimp. However, cell death mechanisms involved must be well understood. It is usually affected by type of cell's death, apoptosis or necrosis pathway, which apoptosis is the most preferred way of cell's death. The mechanisms in apoptosis induction to be targeted are involvement of reactive oxygen species (ROS), extrinsic and intrinsic mechanisms and role of P53. Therefore, if the mechanism of apoptosis induction by *M. oleifera* is well understood and discovered, it will provide potential medicine which is designed to inhibit or induce certain pathways that involved in cancer diseases.

i. ROS mediated signalling pathway

Apoptosis induction is also related with mechanism of reactive oxygen species (ROS) mediated signalling pathway. ROS mediators affect intracellular signalling, caused damage of DNA and indulged epigenetic alterations through the stages of cancer or tumour development, as well as activating apoptotic pathway when ROS is imbalance (Saravanan et al., 2003). In induction of apoptosis, ROS mediated signalling pathway provide mediators in cell cycle progression. If *M. oleifera* is used to induce apoptosis in cancerous cell through ROS mediated signalling pathway, it should has the ability to reduce the overexpression of ROS. This is because, a study was reported that 5-HMF (5-hydroxymethylfurfural, C6H6O3) reduced ROS expression in A375 cells in dose

time dependent suggesting that it inhibits A375 cells growth by manipulating oxygen metabolism in the cell (Zhao et al., 2014).

A study was reported that *M. oleifera* stem bark extracts incorporated in silver nanoparticles (AGNPs) were found to induce apoptosis in human cervical carcinoma cells by increasing ROS generation and its subsequent action. It was suggested that AGNPs increase ROS generation by inhibiting cell's replication (Vasanth et al., 2014). Figure 7 shows example of occurrence when AGNPs are utilized to activate ROS. Caspase family will be activated thus caused programmed cells death. Apoptosis of HeLa cells also occurred through extrinsic and intrinsic pathway when AGNPs were introduced to the cells. Expression of ROS was also responsible in inhibiting A375 cell proliferation through activation of cell cycle arrest (Zhao et al., 2014). Indeed, the study was corroborate with the fact that ROS played an important role to inhibit cancer or tumor progression. Therefore, the mechanism of ROS mediated pathway must be fully understood in broad aspects so that all the signalling that involved could be inhibited by potential chemopreventive agent.

ii. Targeting extrinsic and intrinsic pathway of apoptosis induction

When phytochemicals caused caspase activation, apoptosis can occur by two entry points, which are extrinsic pathway through receptor pathway in plasma membrane and the other entry point is intrinsic pathway through mitochondria pathway in mitochondria (Fulda and Debatin, 2006).

Extrinsic pathway is mediated by death receptors such as CD95, TRAIL and TNF that trigger death signal from plasma membrane through intracellular signalling to inhibit apoptosis (Wu et al., 2005). Some chemopreventive agent developed only to target extrinsic pathway to induce apoptosis such as sarcophine-diol (SD), a chemopreventive agent for skin cancer (Zhang et al., 2009). This is because extrinsic pathway will independently activated p-53 which had indicated good preclinical results to induce apoptosis in various cancer cell lines (Ahskenavi, 2008). Isothiocyanates derived from myrosinase-glucomoringin activation (GMG-ITC) of *M. oleifera* was found to induce caspase-3 dependent apoptosis in multiple myeloma cell (Brunelli et al., 2010). It provided clear proof that hydrolysed glucomoringin from *M. oleifera* can induce apoptosis through extrinsic pathway. But the author claimed that the mechanisms of actions involved are still unknown. However, intrinsic and extrinsic pathways are interconnected with caspase-3 activation where activation of caspase-3 definitely leads to apoptosis (Repnik and Turk, 2010). It was reported that intervention of hormones can contribute to extrinsic apoptosis, as example, testosterones hormone was reported to induce extrinsic apoptosis in prostate cancer when the hormone level is increased (Hongmei, 2012). It is concluded that in balance concentration of the hormone, apoptosis will be inhibited and the hormone also can be an inducer of apoptosis.

Intrinsic pathway is dependent on activation of p53, it is also a caspase independent pathway, because caspase is, not involve literally in the process (Hongmei, 2012). It also can occur by *in vivo* and *in vitro* cell ligands. Studies have focussed on the intrinsic apoptosis to understand the pathways and mechanisms involved so designated drug can be discovered to target apoptosis in inhibiting cancer and other related diseases. Basically, in intrinsic pathway (Figure 8), when anti-cancer agent is introduced into the cell which it is permeable into mitochondria, Bcl-2 and Bax will be expressed. Bcl-2 and Bax are regulator protein in intrinsic pathway which release cytochrome c or apoptosis inducing factor (AIF) then apoptosis will occur.

However, both extrinsic and intrinsic pathways are interconnected. In the study of inducing apoptosis of cancer cells, both pathway gave important beneficiary values. In the study of *M. oleifera* leaf extracts on human tumour cell line, the extract was able to cause a series of morphological changes such as membrane blebbing of the cells, cytoplasmic membrane shrinkage, loss of contact with neighboring cells, and apoptotic body formation which are the features to indicate apoptotic cell's death (Sreelatha et al., 2011). The morphological changes are caused by caspase substrate cleavage which is involved in apoptosis pathways. The author also reported that *M. oleifera* extracts caused the tumor cell to increase the permeability to propidium-iodide (PI) staining that also displayed nuclear shrinking, DNA condensation and fragmentation. It determined that apoptosis induced by *M. oleifera* leaf extracts were involving intrinsic and extrinsic pathways. Therefore, from the studies, it also indicated that *M. oleifera* can provide promising anti-cancer drug from natural sources that induced apoptotic cell body in cancer cells.

iii. Role of p53 in apoptosis induction

P53 is a tumor suppressor that plays an important role in inducing apoptosis that acts as a major key player in cellular response and affects the mitochondrial intrinsic apoptosis pathway (Yee and Vousden, 2005; Zhang et al., 2013). It is found that, in more than half human cancer diseases occurred, exhibited inactivation or lost function of p53 (Kundu et al., 2014). Therefore, it is important to study the ability of compound to activate p53 in order to induce apoptosis. A report stated that phytochemicals can induce apoptosis by activating p53 in ovarian cancer cells study (Luo et al., 2011). The expression of p53 protein in the cell was analysed by western blotting where the result showed that the phytochemicals increased p53 protein expression. P53 pathway involved in inducing apoptotic in activating caspase 9 pathway (Ashkenazi 2008; Patel et al., 2014). Luo et al. (2011) claimed that the study provided understanding of mechanisms involved in p53 pathway. So, it can be applied by using other phytochemicals such as glucosinolates from *M. oleifera*. However, it was reported that the phytochemicals used in the study cannot differentiate between normal and cancerous cell as it increased p53 protein in both type of the cells. It is important for the compound to be only cytotoxic towards cancer cell not the normal cell. *M. oleifera* leaf extracts were found to maintain 70-90 % of cell's viability in normal cell (Ghosh, 2013). This indicates that *M. oleifera* exhibit good potential in targeting p53

pathway in inducing apoptosis.

Inhibition of tumor angiogenesis by *M. oleifera*

Inducing tumor angiogenesis is one of the six hallmarks of cancer progression proposed by Hanahan and Weinberg (2011). Tumorigenesis or tumor angiogenesis can be determined through expression of NF-κB protein in cancer cell (Kundu et al., 2014). 4(α-L-rhamnosyloxy)-benzyl isothiocyanate) (GMG-ITC) produced from glucomoringin of *M. oleifera* was found to inhibit NFκB activity in RAW-NFκB cells that indicates inhibition of tumor angiogenesis (Brunelli et al., 2010). It was also reported to reduce tumor growth in mouse with A2780 ovarian cancer (Brunelli et al., 2010). It gave concrete proof that the compound from *M. oleifera* works well in inhibition of NFκB activity through *in vivo* and *in vitro* mechanisms. However tumorigenesis inhibition can also cause by other factors. So, the pathways and transcriptors involved in *in vivo* study of NFκB activity should be further studied to determine whether it was caused by inhibition of NFκB activity which it is not mentioned in most study. NFκB transcriptors are also responsible in many cellular signalling pathways, so it is important to study *M. oleifera* effect to be used in inhibiting tumour angiogenesis for cancer chemoprevention.

Synergistic effect of *M. oleifera* and its safety

The uses of *M. oleifera* compound as commercial medicine for chemopreventive agent has not been developed yet. However, it showed positive synergistic effect in increasing cytotoxicity of doxorubicin against cervical cancer cell line (HeLa) (Adam et al., 2012). Doxorubicin is one of the cancer therapy drugs derived from chemical's semi synthesis. The combination of *M. oleifera* and doxorubicin were resulted to enhance apoptosis induction. Interestingly, the author also stated that the mechanisms involved by the combination of *M. oleifera* and doxorubicin were still unclear. Both chemical compounds may have ability to induce apoptosis, so when both were combined, they exhibited stronger effect. The findings of the study suggested that by combining *M. oleifera* extract to cancer therapy drug available, it may decrease toxic side effect of the drug as well as enhancing chemopreventive effect of the drug. *M. oleifera* extracts had long been used in traditional medicine (Nadkarni, 2009; Saha, 2013). This proved that the plant is safe to consume and taken as edible vegetable.

Conclusion

In conclusion, most of the *M. oleifera* extracts had exhibited chemopreventive effect in various cancer studies. It is suggested that the mechanisms involved in chemoprevention of *M. oleifera* must be studied extensively. As in coming years, cancer diseases is expected to increase if it is not under controlled. *M. oleifera* can be a good source to develop chemopreventive agent in controlling the diseases by targeting it to inhibit several major mechanisms in cancer process, especially in some less discovered mechanisms. More *in vivo* studies must be done to provide more information of its effects to cancer cell process and development.

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