Food Research 7 (6) : 54 - 63 (December 2023)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

The cytotoxicity activity of Borneo-cultivated mulberry against breast cancer cell (MCF-7): the influence of maturity stages and extraction solvents

[1]Chen, C., [2]Mokhtar, R.A.M., [2]Kamaruzaman, K.A., [2]Wei, G.T.Y. and [1,*]Nor Qhairul Izzreen, M.N.

[1]*Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Kota Kinabalu 88400, Sabah, Malaysia*
[2]*Biotechnology Research Institute, Universiti Malaysia Sabah, Kota Kinabalu 88400, Sabah, Malaysia*

Article history:
Received: 14 October 2022
Received in revised form: 25 December 2022
Accepted: 21 March 2023
Available Online: 29 November 2023

Keywords:
Anticancer activity,
Breast cancer,
Different maturities,
Different extraction solvents,
Morus alba Linnaeus

DOI:
https://doi.org/10.26656/fr.2017.7(6).519

Abstract

Breast cancer is one of the leading causes of death in a part of Borneo, which is Sabah, Malaysia, and worldwide. The anticancer activity of white mulberry (*Morus alba* Linnaeus) has been widely reported. However, cultivation locations, maturity levels, and extraction solvents could influence their phytochemical and pharmacological activities. The lack of anticancer investigation has hampered the development and potential use of Borneo-grown mulberry as an anticancer agent or source. This study investigated the cell cytotoxicity activity of Malaysia-grown mulberry at two maturity stages (fruits: brackish black fully ripe and red mature; leaves: young and mature) extracted using 70% (v/v) methanol, 60% (v/v) ethanol, and 65% (v/v) acetone, against a human breast cancer cell line (MCF-7). As a result, fruits demonstrated maturity-dependent cytotoxicity decrement as the red mature fruits in 70% (v/v) methanol exerted the strongest cytotoxicity (IC_{50} = 26.83 mg/mL). Meanwhile, the cytotoxicity of leaves revealed maturity-dependent increment as mature leaves in 60% (v/v) ethanol exhibited the strongest cytotoxicity against MCF-7 (IC_{50} = 2.45 mg/mL). Their cytotoxicity is possibly correlated to the anticancer-possessing phenolic acids and flavonoids in fruits; and the anticancer-possessing alkaloids and terpenes in leaves. Overall, Sabah-grown mulberry possesses substantial cytotoxicity against MCF-7, suggesting its anticancer potential as a complementary treatment for breast cancer.

1. Introduction

Morus alba Linnaeus, also known as white mulberry, is one of the medicinal plants with renowned therapeutic effects. The multi-functionality of mulberry fruits, leaves, roots, branches and barks have been well documented in the Chinese Pharmacopoeia and British Herbal Pharmacopoeia (Khan *et al.*, 2013; Younus *et al.*, 2016; He *et al.*, 2018). Mulberry contains abundant metabolites, including macronutrients, micronutrients, phenolic acids, flavonoids, flavonols, anthocyanins, vitamins and volatile aromatic compounds (Sánchez-Salcedo *et al.*, 2015a; Sánchez-Salcedo *et al.*, 2015b). Its fruits and leaves contain significant amounts of phytochemical compounds, including ferulic acid, gallic acid, chlorogenic acid, protocatechuic acids, quercetin, rutin, apigenin, and others, that have been proven to exhibit excellent antioxidative activity. The potent antioxidant activity of mulberry fruit was seen from DPPH (IC_{50} of 0.518 mg/mL), FRAP (IC_{50} of 0.522 mg/mL) and lipid peroxidation inhibition in the liver (45.5%), microsomes (42.8%), and mitochondria (39.4%) (Sánchez-Salcedo *et al.*, 2015a; Natić *et al.*, 2015). While the leaves had FRAP activity of 3.35–4.37 mg GAE/g DW and ascorbic acid equivalent antioxidant activity of 5.08–9.70 mg ascorbic acid/g DW (Eric *et al.*, 2012). Aside from being antioxidative, the compound richness of mulberry is also the main contributor to its various pharmacological effects, including its anticancer effects against various cancers (Dat *et al.*, 2010).

Cancer is the second leading cause of death worldwide. It is a disease characterized by the abnormal growth rate of body cells that can occur in any tissue or organ and is capable of spreading to other organs. According to World Global Cancer Incidence, Mortality and Prevalence (GLOBOCAN) 2020, a total of 19.3 million new cancer cases and 10 million cancer deaths, with 50.6 million 5-year prevalent cases, were publicized (Sung *et al.*, 2021). Moreover, an increase to a total of 28.4 million new cancer incidents and 16.2 million cancer-related deaths is estimated in 2040. In Malaysia, cancer is the top four most prominent cause of death. In Malaysia, the Malaysia National Cancer Registry

*Corresponding author.
Email: *qhairul@ums.edu.my*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

(MNCR) 2012-2016 reported a total of 115,238 new cancer cases, with 44.7% among men and 55.3% among women (Azizah *et al.*, 2019). Also, according to the Malaysia Ministry of Health (MOH) hospitals, an increase of 2.64% in cancer-related deaths has occurred from 2004 (9.54%) to 2019 (12.18%) (Ministry of Health Malaysia, 2021). Out of these cancer cases, breast cancer was reported as the highest cancer case in Malaysia, with a rate of 19% in MNCR 2012-2016 and 32.9% in Malaysia GLOBOCAN 2020 (Azizah *et al.*, 2019; Sung *et al.*, 2021). In Sabah alone, the western state of Malaysia, MNCR 2012-2016 recorded a total of 8,818 cancer incidences with a 45.4% occurrence in men and 54.6% in women, and breast cancer accounted for the highest cancer incidences (13.8%) in Sabah (Azizah *et al.*, 2019). Details indicate a total of 1206 (25.1%) breast cancer cases in Sabahan women and only 12 (0.3%) cases in Sabahan men. Moreover, a study in Sandakan City, Sabah has identified a total of 115 breast tumour cases from 2016–2018, with invasive ductal carcinoma (4.82%) as the highest case, followed by invasive lobular carcinoma (2.9%) (Wong *et al.*, 2021).

Free radicals, especially reactive oxygen species (ROS), are the hallmark of cancer's aetiology (Ríos-Arrabal *et al.*, 2013). It is due to their ability to damage and mutate DNA through the rearrangement of DNA sequence, miscoding DNA lesions, amplification of genes, modification of bases, breakage of single or double-strand, and activation of oncogenes (Ríos-Arrabal *et al.*, 2013; Pourahmad *et al.*, 2016). These events can cause deleterious effects and loss of cell cycle control, inducing the initiation and development of several cancers. Moreover, the extra ROS that is created through the metabolism of carcinogens (Pourahmad *et al.*, 2016), macrophages, mitochondria, and peroxisomes, might play a role in cancer proliferation (Pucci *et al.*, 2019). Hence the importance of antioxidants to maintain redox homeostasis. Endogenous antioxidants aside, exogenous antioxidants are needed to boost the body's homeostasis, and natural antioxidants are gaining greater attention due to their safer side effects than synthetics. Plant phytochemicals are excellent natural antioxidants. Several clinical trials have been conducted on phenolic acids, flavonoids, alkaloids and vitamins for their preventive and therapeutic drug abilities (Singh *et al.*, 2016; Chikara *et al.*, 2018). This is due to their proven antioxidant, and anti-inflammatory properties as well as their significant *in vitro* and *in vivo* anti-proliferative and pro-apoptotic properties (Chikara *et al.*, 2018). As a result, some of these compounds have been introduced as complementary cancer therapies.

In the East of Malaysia, a three-hectare mulberry plantation at Tudan village, Tuaran, Sabah is being underutilized. Previous studies showed that the fruit and leaves of this Sabah-cultivated mulberry contain high levels of phytochemicals and antioxidant activity that are influenced by maturity levels and extraction solvents (Centhyea *et al.*, 2020; Chen *et al.*, 2022). As reported in various studies, mulberry contains an abundant amount of bioactive compounds whose anticancer activity has been validated against various cancers (Dat *et al.*, 2010). However, this Sabah-grown mulberry has not been subjected to any anticancer studies, which hampered its growth and possible application as an anticancer source or agent. Accordingly, variations in species, development levels, cultivation locations, environments, and handling or processing techniques could influence the biosynthesis of metabolites in plants (Radojkovića *et al.*, 2012; Lee and Hwang, 2017; Li *et al.*, 2020). These variations would consequently influence the chemical and biological activity of plants, including their anticancer activity. For example, a study on Sabah tea leaves under the influence of different maturity stages, fermentation stages, and extraction solvents revealed that the ethanol (EtOH) extracted green tea exerted higher cytotoxicity against human lung (A549) (IC_{50} = 61.0), breast cancer (MDA-MB-231) (IC_{50} = 34.0), and cervical cancer cell lines (HeLa) (IC_{50} = 30.0), compared to the black tea, compost, and waste tea leaves (Izzreen *et al.*, 2020). In a different study, the n-hexane and dichloromethane fractions of mulberry fruits demonstrated the highest cytotoxicity against colon (HCT116) (100%) and breast cancer cells (MCF-7) (99.4%) compared to other solvent extracts (El-Baz *et al.*, 2017). Meanwhile, a study on mulberry leaves showed that the 50% (v/v) methanol (MeOH) and 100% MeOH leaf extracts exerted the best inhibition of hepatocellular carcinoma's (HepG2) proliferation via NF-κB suppression and biomarker modulation (Fathy *et al.*, 2013). Hence, the distinction between maturity stages and extraction solvents is important to obtain the optimal anticancer activity of a plant. Therefore, in this study, the local Sabah highland-cultivated mulberry fruit and leaves at different maturity levels (fruits: red mature (RF) and brackish black fully ripe (BF); leaves: mature (ML) and young (YL)) and extracted in different solvents (70% (v/v) methanol (MeOH), 60% (v/v) ethanol (EtOH), and 65% (v/v) acetone) were analyzed for their cytotoxicity activity against MCF-7, which is the highest cancer incident in Sabah, Malaysia, and worldwide. This research will be the first step to finding out the anticancer potential of Sabah-cultivated mulberry in the pharmaceutical industry.

2. Materials and methods

2.1 Sample collection and preparation

Both the mulberry (*Morus alba* Linnaeus) fruit and

https://doi.org/10.26656/fr.2017.7(6).519

leaf samples were harvested from Tudan Village, Tuaran, Sabah, East Malaysia (Borneo). The fruits at maturity index 4 (red and mature) and 5 (brackish black and fully ripe) (Lee *et al.*, 2016) were collected in September and October of 2018 and 2019. For the leaves, young (the 1st to 4th leaves from the shoot) and mature leaves (the 5th to 8th leaves from the shoot) (Wulandari *et al.*, 2019) were collected between July and August 2020.

2.2 Preparation of mulberry extracts

The collected brackish black fully ripe fruits (BF), red ripe fruits (RF), young leaves (YL), and mature leaves (ML) were washed, frozen in a -80°C freezer (New Brunswick Scientific U410, Germany) for 24 hrs, and freeze-dried (Labconco, USA) at -40°C and 0.5 Pa for 72 hrs. Next, samples were blended to powder using a laboratory blender (Waring 8010S, USA), set to 18,000 rpm for 5 mins. The powdered mulberry fruit and leaves were respectively extracted using 70% (v/v) MeOH (Tan *et al.*, 2015), 60% (v/v) EtOH (Radojkovića *et al.*, 2012), and 65% (v/v) acetone (Jeszka-Skowron *et al.*, 2014), with a ratio of 1: 30 (m/v) at 60°C in a shaking water bath (Daihan MaXturdy 30, Korea), set at 120 rpm for 4 hrs (Radojkovića *et al.*, 2012; Kim *et al.*, 2014). The samples were filtered through Whatman paper No.1 and the filtrates were rotary evaporated at 40°C (Heidolph Laborota 4000, Germany) until dry. Samples were further dried using a freeze-dryer (Labconco, USA) at -40°C and 0.5 Pa for 48 hrs, then re-dissolved in sterilized distilled water (dH_2O).

2.3 Cell culture

The human breast cancer (MCF-7) cell line (Elabscience Biotechnology Inc., USA) was cultured in a T25 flask with 5 mL of complete media consisting of Dulbecco's modified Eagle's medium (DMEM) (Nacalai Tesque, Japan), 10% fetal bovine serum (FBS) (Tico Europe, Netherlands) and 1% of 100 U/mL of penicillin-streptomycin mixed solution (Nacalai Tesque, Japan). The cells were incubated for growth at a 37°C incubator supplemented with 5% purified carbon dioxide (CO_2). Cells were sub-cultured upon reaching 70%-80% confluence.

2.4 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay

The MTT assay was conducted according to Deepa *et al.* (2013) with modifications. A total volume of 90 µL of cells and media mixture was seeded into a 96-well microplate to culture 1×10^4 cells/well. The MCF-7 cells were cultured for 24 hrs in a 37°C incubator supplemented with 5% purified CO_2 to reach 60% confluence. After that, the old media was replaced with a fresh 90 µL of complete media and the cells were treated with mulberry fruit and leaf extracts for another 24 hrs in a 37°C incubator supplemented with 5% purified CO_2. Next, 80 µL of 0.5 mg/mL MTT (Nacalai Tesque, Japan) dissolved in 1X PBS was added to each well and incubated for 4 h in a 37°C incubator supplemented with 5% purified CO_2. The supernatant was then removed from each well and 100 µL of dimethyl sulfoxide (DMSO) (Nacalai Tesque, Japan) was added. The mixture was then incubated for 20 mins before being measured at 570 nm via the Tecan Infinite M200 plate reader (Tecan, Switzerland). The final results were given in the form of IC_{50} by plotting the graft percentage (%) of live MCF-7 cells against the fruit and leaf concentrations via the formula (1).

$$\text{Cell viability (\%)} = [A_1/A_0] \times 100 \quad (1)$$

Where A_0: abs of control: 1×10^4 cells/well + 10 µL of sterilized dH_2O and A_1: abs of sample: 1×10^4 cells/well + 10 µL of BF/RF/YL/ML in sterilized dH_2O.

2.5 Data analysis

All experiments were performed in triplicate. All data is expressed as the mean ± standard deviation (SD). A two-way Analysis of Variance (ANOVA) (IBM SPSS Statistics 23.0, IBM Corp., Armonk, New York) was performed to determine the effect of two independent factors (maturation stages and extraction solvents) on dependent factors (cytotoxicity against MCF-7). Upon the significant difference ($p < 0.05$) of two-way ANOVA, a one-way ANOVA and Tukey's HSD Comparison Test at a 95% significance level ($p < 0.05$) were conducted to see the significant difference among samples (IBM SPSS Statistics 23.0, IBM Corp., Armonk, New York).

3. Results and discussion

3.1 The cytotoxicity of mulberry fruits against MCF-7

The cytotoxicity of mulberry fruits against MCF-7 is shown in Table 1. Mulberry fruits showed a maturity-dependent increment of cytotoxicity, indicating the stronger cytotoxicity of RF than BF against MCF-7. RF showed 64.2% stronger cytotoxicity activity against MCF-7 (IC_{50} = 26.83–31.41 mg/mL) than BF (IC_{50} = 77.06–85.73 mg/mL). This difference can also be seen from Figure 1 which shows the condition of MCF-7 after the treatment of BF and RF. The two-way ANOVA revealed a significant relationship ($p < 0.05$) between ms*es on the cytotoxicity of mulberry fruits against MCF-7.

The obtained data is seen to be more cytotoxic than the cytotoxicity of mulberry fruits in Gug (2012) (IC_{50} =

https://doi.org/10.26656/fr.2017.7(6).519

Table 1. The cytotoxicity of mulberry fruits and leaves against MCF-7. The result of two-way ANOVA is shown for the interaction of the two factors: maturation stages (ms) and extraction solvent (es).

Analysis	Result in Different Maturities and Different Solvents[1]						Two-Way ANOVA ms*es (*p*-Value)
	MeOH	EtOH	Acetone	MeOH	EtOH	Acetone	
Fruits MTT (IC_{50})	BF			RF			
	81.25±1.86^{ab}	85.73±4.39^{a}	77.06±3.1^{b}	26.83±1.47^{c}	29.05±0.92^{c}	31.41±3.41^{c}	< 0.05
Leaves MTT (IC_{50})	YL			ML			
	10.62±0.42^{c}	12.38±0.68^{b}	14.69±0.40^{a}	4.33±0.91^{d}	2.45±0.25^{e}	4.55±0.11^{d}	< 0.001

Values are presented mean±SD (n = 6). Values with different superscripts are statistically significantly different (p<0.05). If a significant interaction effect was found between ms*es (p < 0.05), one-way ANOVA on the combination factor of both effects was run. The interaction effect is the most important effect.BF: Brackish black fully ripe fruits, RF: Red mature fruits, YL: Young leaves, ML: Mature leaves, MeOH: 70% (v/v) methanol, EtOH: 60% (v/v) ethanol, Acetone: 65% (v/v) acetone.

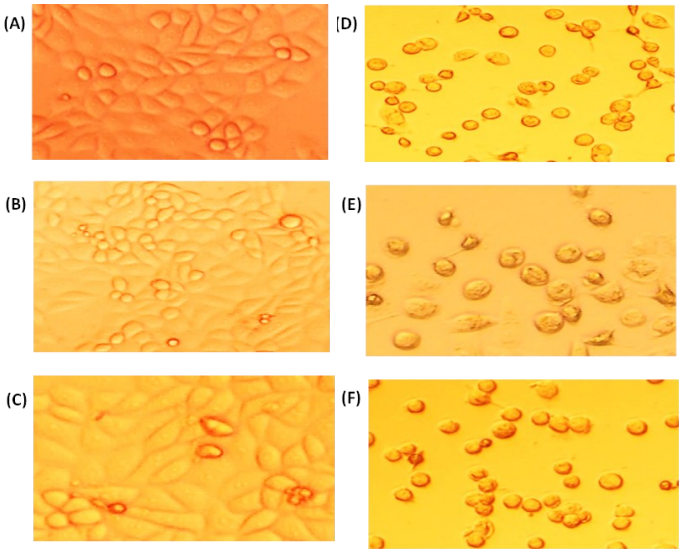

Figure 1. MCF-7 after treated with BF and RF at 50 mg/mL under 4× magnification using inverted microscope. (A) BF 70% (v/v) methanol; (B) BF 60% (v/v) ethanol; (C) BF 65% (v/v) acetone; (D) RF 70% (v/v) methanol; (E) RF 60% (v/v) ethanol; (F) RF 65% (v/v) acetone.

295.29 mg/mL). Upon treatment with mulberry fruits, changes in cell shape from polygonal-spindle shape to round shape were observed, indicating the possible apoptotic or necrosis reaction. A higher amount of these possibly-apoptotic cells was seen under the treatment of RF compared to BF at the same concentrations (50 mg/mL). The higher cytotoxicity of RF is speculated to be due to the greater amounts of chlorogenic acid and rutin quantified via UHLPC in our previous study (Chen *et al.*, 2022). Chlorogenic acid has been shown to kill MCF-7 in a dose-dependent manner (IC_{50} = 52.5 µg/mL) and to induce mitochondrial pathway apoptosis (Deka *et al.*, 2017). Chlorogenic acid also translocates 12.1% of protein kinase C from the cytosol to the plasma membrane and induces G1-phase arrest of MCF-7 with a reduction in S-phase. Similar data is observed in blueberries, whose anticancer ability declined with the declining quinic acid, chlorogenic acid, methylsuccinic acid, malic acid, and oxoadipic acid across the fruits' maturity levels (Das *et al.*, 2022). Meanwhile, rutin is able to inhibit MCF-7 proliferation (IC_{50} = 46.09 µM) and induce apoptosis via G2/M cell arrest, up-regulation of the tumour suppressor genes; p53 and PTEN (by 1.52 and 13.47-fold, respectively), and cell cycle-associated genes; p21 and CDK1 (by 1.28 and 1.15 fold, respectively) (Hasani *et al.*, 2018). Hence, the stronger anticancer ability of RF can be associated with its higher level of chlorogenic acid and rutin than BF.

In addition, studies have reported the higher presence of other anticancer-possessing phenolics and compounds in less ripe fruits. Examples of these compounds are protocatechuic acid, caffeic acid, ferulic acid and ellagic acid (Lee and Hwang, 2017). According to various studies, ferulic acid has an IC_{50} of 75.4 g/mL against MCF-7 and induces apoptosis via caspase-3 and -9 elevation (ElKhazendar *et al.*, 2019), whereas ellagic acid induces cell cycle arrest and apoptosis via the transforming growth factor-β (TGF-β)/Smad3 signalling pathway (Chen *et al.*, 2015). Likewise, Lee *et al.* (2016) discovered higher levels of aconitate, phenolics (neochlorogenic acid, cryptochlorogenic acid, and chlorogenic acid), and vitamins (δ-tocopherol and ascorbic acid) in red mature mulberry fruits than in brackish black fully ripe fruits. The cytotoxicity of ascorbic acid was seen through its dose-dependent MCF-7 inhibition and ability to enhance the release of lactate dehydrogenase to induce MCF-7 apoptosis (Ganash, 2021).

Besides, the mature mulberry fruits were also revealed to contain a higher concentration of polyunsaturated fatty acids (PUFA) than the fully ripe fruits (Lee *et al.*, 2016; Jelled *et al.*, 2017). Reports showed that docosahexaenoic acid (DHA) can reduce the size of breast tumours in mice, increase the expression of the BRCA1 tumour suppressor gene by 60%, and induce apoptosis by activating peroxisome proliferator-activated receptors γ (PPARγ) (Berquin *et al.*, 2008). Meanwhile, omega-3 PUFA have been shown to have anticancer activity, improve chemotherapy efficacy, and reduce cancer incidence and mortality (Berquin *et al.*, 2008;

https://doi.org/10.26656/fr.2017.7(6).519

Jóźwiak *et al.*, 2020). Therefore, the higher cytotoxicity activity of RF against MCF-7 in this study could be associated with their higher content of phenolic compounds, including chlorogenic acid and rutin, vitamins, and PUFA, that contain strong anticancer activity.

For the assessment among solvents, some inconsistency was seen in BF and RF. In BF, the 65% (v/v) acetone extract exhibited the highest cell inhibitory activity against MCF-7 (IC_{50} = 77.06 mg/mL), which was 5.16% better than 70% (v/v) MeOH extracts and 10.1% better than 60% (v/v) EtOH. This data is consistent with its highest quantified chlorogenic acid value (4.49 mg CGAE/g DW) in our previous study (Chen *et al.*, 2022). However, in RF, the 70% (v/v) MeOH extract exerted the highest inhibitory action (IC_{50} = 26.83 mg/mL), which was 7.6% better than 60% (v/v) EtOH and 14.6% better than 65% (v/v) acetone extracts. This data is also consistent with its highest quantified rutin value (4.93 mg RE/g DW) in our previous study (Chen *et al.*, 2022). Based on this, it can be concluded that chlorogenic acid and rutin have a positive correlation with the cytotoxicity activity of fruits against MCF-7. Hence, the anticancer activity of the fruit extracts depended on the solubility rate of chlorogenic acid and rutin in the solvents.

The variation of anticancer ability among the three solvents could also be influenced by the solubility rate of other anticancer compounds. For instance, ellagic acid was reported to be sparingly soluble in alcohol such as methanol (671 µg/mL) but poorly soluble in water (9.7 µg/mL) (Bala *et al.*, 2006). This is based on the higher ellagic acid content found in the MeOH extract of the chestnut shell (12.57 mg/g), compared to its EtOH extract (4.07 mg/g) (Jung *et al.*, 2016). Next, *p*--Hydroxybenzoic acid revealed better solubility in MeOH, followed by EtOH, acetone and other lower polarity solvents (Gracin and Rasmuson, 2002). A study of protocatechuic acid also revealed its greater solubility in MeOH than acetone, evidenced by its higher solvation free energy and higher number of formed hydrogen bonds (Gurina *et al.*, 2017). Further, it was reported that ascorbic acid has better solubility in more polar solvents, indicating the solubility order of: Water > MeOH > EtOH > Acetone (Nemdili *et al.*, 2022). Therefore, the higher cell inhibition activity of RF 70% (v/v) MeOH could be due to the higher solubility of ellagic acid, *p*-Hydroxynamic acid, and ascorbic acid in it.

3.2 The cytotoxicity of mulberry leaves against MCF-7

Table 1 reveals the cytotoxicity of mulberry leaves against MCF-7 in a maturity-dependent increment manner. ML showed 70.0% higher cytotoxicity (IC_{50} = 2.45–4.55 mg/mL) than YL (IC_{50} = 10.62–14.69 mg/mL) against MCF-7. This difference can also be seen from Figure 2 which shows the condition of MCF-7 after being treated with YL and ML. The two-way ANOVA revealed a significant relationship ($p < 0.05$) between ms*es on the cytotoxicity of mulberry leaves against MCF-7. This finding was supported by Hyun *et al.* (2013), which mature and senescent yellow Korean *Dendropanax* leaves had greater cell inhibitory and anti-migratory effects against hepatocellular carcinoma cells (Huh-7) than the non-senescent leaves at sample concentrations of 100 µg/well and 200 µg/well.

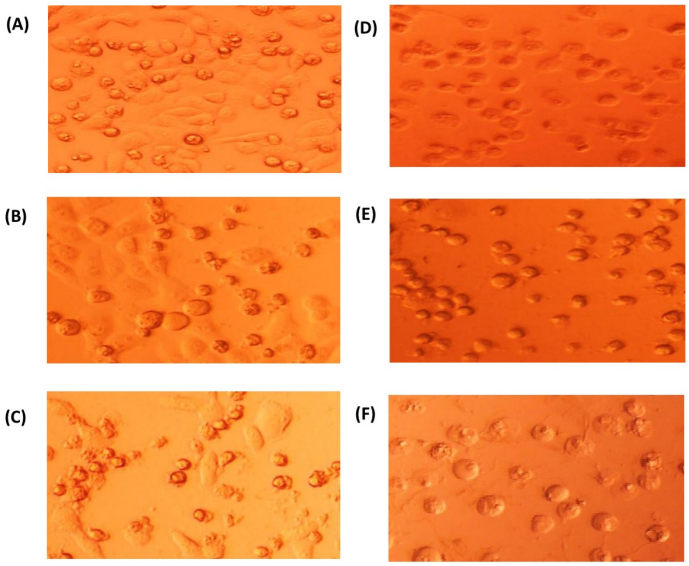

Figure 2. MCF-7 after treated with YL and ML at 12.5 mg/mL under 4× magnification using inverted microscope. (A) YL 70% (v/v) methanol; (B) YL 60% (v/v) ethanol; (C) YL 65% (v/v) acetone; (D) ML 70% (v/v) methanol; (E) ML 60% (v/v) ethanol; (F) ML 65% (v/v) acetone.

Similarly in leaves, changes in MCF-7 cell shape from polygonal-spindle shape to round shape were observed upon treatment with YL and ML, indicating a possible apoptotic or necrosis reaction. Moreover, a higher amount of these possibly-apoptotic cells was seen under the treatment of ML compared to YL at the same concentration (12.5 mg/mL). The better cell inhibitory effect of ML against MCF-7 is speculated to be due to the higher quantity of 1-Deoxynojirimycin (DNJ) in mature mulberry leaves (0.16–0.27 mg DNJ/g leaves) than in the young leaves (0.07–0.08 mg DNJ/g leaves) (Wulandari *et al.*, 2019). One study revealed that 0.02-0.1% of DNJ-diets have significantly reduced the incidence of colorectal cancer in mice and dose-dependently suppressed the growth of cancer (Yamamoto *et al.*, 2017). Meanwhile, a derivative of DNJ has down-regulated the expression of cyclooxygenase 2 and arrested the cell cycle of MCF-7 at the S phase (Zhang *et al.*, 2021). This derivative compound also inhibited the migration of MCF-7 and induced cell apoptosis via a mitochondrial-mediated pathway. Both DNJ and the derivative decreased the

https://doi.org/10.26656/fr.2017.7(6).519

expression of anti-apoptotic Bcl-2 but increased the expression of pro-apoptotic Bax (Yamamoto *et al.*, 2017; Zhang *et al.*, 2021). All of these have validated the anticancer activity of DNJ. Hence, the stronger cytotoxicity of ML in this study could be due to its higher DNJ value.

Wulandari *et al.* (2019) also reported a higher presence of simple terpenes, such as phytol and limonene, in mature mulberry leaves (8.16% and 6.33%, respectively) than in young leaves (0.69% and 1.12%, respectively). One study reported that phytol showed the strongest cytotoxicity against MCF-7 (IC_{50} = 8.79 µM) compared to the cervical, colon, lungs and other cell lines (Pejin *et al.*, 2014). Furthermore, a daily 8 g/m^2 dose of D-limonene showed a partial positive response in a pharmacokinetics study of advanced breast cancer (Sun, 2007). This diet has managed to stabilise the axillary and supraclavicular lymph node carcinoma and reduce more than 50% of the supraclavicular lymphadenopathy before the spread of cancer to the bones after 11 months of treatment. Besides, Wulandari *et al.* (2019) also identified 1.87% of citronellal in mature leaves but not in young leaves. Citronellal was proven to exert cytotoxicity (IC_{50} = 1.41 nM) and reduce cell proliferation and migration of MDA-MB-231 cells (Ho *et al.*, 2020). Citronellal also arrests cells at the G2/M phase and induces 13.92% apoptosis via a caspase-dependent pathway, increment of the Bax gene, and reduction of the Bcl-2 gene. Based on the strong anticancer ability of phytol, limonene, and citronellal, the stronger anticancer ability of ML in this study might be due to their higher presence in mature mulberry leaves than in young leaves.

In addition, the anticancer properties of mulberry leaves and their anticancer-contributing compounds have been extensively reviewed by Chan *et al.* (2020). However, based on the lower bioactive compounds, chlorogenic acid, and rutin content of ML in our previous study (Chen *et al.*, 2022), as well as the higher phenolic acid and flavonoid compounds in He *et al.* (2019), it could be speculated that the anticancer ability of leaves has a better correlation with other secondary metabolites such as alkaloids and terpenes instead of phenolic acid and flavonoid compounds.

For the assessment among solvents, inconsistency of data was also seen in the leaves (Table 1). In YL, the 70% (v/v) MeOH extract revealed the highest inhibition activity against MCF-7 (IC_{50} = 10.69 mg/mL). Meanwhile, in ML, the 60% (v/v) EtOH exhibited the highest cell inhibition against MCF-7 (IC_{50}= 2.45 mg/mL). The obtained data from ML is similar to the higher cytotoxicity of EtOH-extracted *Cichorium intybus* against MCF-7 than the MeOH extract at 250 ppm and 750 ppm (Kandil *et al.*, 2019). In contrast to fruits, only the ML 60% (v/v) EtOH demonstrated consistency with the highest chlorogenic acid content (8.78 mg CGAE/g DW) in our previous study (Chen *et al.*, 2022). Nonetheless, its lower chlorogenic acid value than YL (8.93–30.73 mg CGAE/g DW) indicates its low correlation.

On the other hand, the different cytotoxicity of solvents is correlated to the presence of compounds possessing anticancer properties. For instance, Themelis *et al.* (2018) mentioned that the high polarity of DNJ has allowed its extraction using various solvents, including water, electrolytes water, and/or their mixture with MeOH or EtOH. In fact, the 70% (v/v) EtOH in their study extracted the highest DNJ value (84%) and was used for their subsequent assays. Next, the preferable solubility of phytol in EtOH was seen by the higher amount of phytol in the chromatographic separation of the EtOH fraction than the MeOH extract (Chakraborty *et al.*, 2021). Furthermore, D-limonene is reported to be partially soluble in water but miscible in hydrocarbons and alcohol solvents, except MeOH (Ngema *et al.*, 2012). Therefore, the interchanging miscibility of these compounds in both EtOH and MeOH could be the reason for the highest cytotoxicity of ML at 60% (v/v) EtOH, followed by 70% (v/v) MeOH, and 65% (v/v) acetone. Unfortunately, further discussion of other possible compounds has been hampered by the lack of studies on the presence of anticancer compounds in different maturities of mulberry leaves.

4. Conclusion

In conclusion, Sabah-cultivated mulberry possesses a substantial cytotoxic activity against the human breast cancer cell line (MCF-7), which is significantly affected by the maturity levels and extraction solvents. The cytotoxicity of mulberry fruits occurs in a maturity-dependent decrement manner, as the red mature mulberry fruits exert higher cytotoxicity compared to the brackish black fully ripe fruits. Among the solvents in fruits, the 70% (v/v) methanol of red mature fruits displayed the strongest cytotoxicity. Meanwhile, the cytotoxicity of mulberry leaves against MCF-7 shows a maturity-dependent increment. The mature leaves of mulberry exhibit stronger cytotoxicity than the young leaves and, among the solvents, the 60% (v/v) ethanol of mature leaves exhibited the strongest cytotoxicity against MCF-7. The cytotoxicity of fruits is possibly correlated to their anticancer-possessing phenolic acids and flavonoids, whereas the cytotoxicity of leaves is conceivably correlated to anticancer-possessing alkaloids and terpenes. The cytotoxicity variation of the three

https://doi.org/10.26656/fr.2017.7(6).519

solvent extracts is due to the rate of solubility of anticancer-possessing compounds in different solvents. Overall, the red mature fruits and mature leaves of the mulberry are significantly more cytotoxic against MCF-7. This indicates their potential anticancer activity and functionality as a complementary treatment for breast cancer. Nevertheless, the screening of compounds responsible for their anticancer activity, apoptotic reaction and cytotoxicity against normal cell lines should be the focus of future studies before delving into their *in vivo* and clinical trials.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This research was funded by Universiti Malaysia Sabah, grant numbers SDK0243-2020 and GUG0501-2/2020. The authors would like to express their gratitude to Dr. Nurul Elyani Mohamad for her assistance in providing technical support. Additionally, special thanks to Gao Peiru for proofreading this manuscript.

References

Azizah, A., Hashimah, B., Nirmal, K., Zubaidah, A.R.S., Puteri, N.A., Nabihah, A., Sukumaran, R., Balqis, B., Nadia, S.M.R., Sharifah, S.S.S., Rahayu, O., Alham, O.N. and Azlina, A.A. (2019). Malaysia National Cancer Registry Report (MNCR) 2012 - 2016. Retrieved on September 14, 2021 from Ministry of Health Malaysia Website: https://drive.google.com/file/d/1BuPWrb05N2Jez6sEP8VM5r6JtJtlPN5W/view.

Bala, I., Bhardwaj, V., Hariharan, S. and Kumar, M.R. (2006). Analytical methods for assay of ellagic acid and its solubility studies. *Journal of Pharmaceutical and Biomedical Analysis,* 40(1), 206-210. https://doi.org/10.1016/j.jpba.2005.07.006

Berquin, I.M., Edwards, I.J. and Chen, Y.Q. (2008). Multi-targeted therapy of cancer by omega-3 fatty acids. *Cancer Letters,* 269(2), 363-377. https://doi.org/10.1016/j.canlet.2008.03.044

Centhyea, Fadhilah, M. and Izzreen, M.N.Q. (2020). Determination of the Antioxidant Activity and Bioactive Compounds of Mulberry Fruit Extracts. IOP Conference Series: Earth and Environmental Science, 736, 012011. https://doi.org/10.1088/1755-1315/736/1/012011

Chakraborty, S., Majumder, S., Ghosh, A., Saha, S. and Bhattacharya, M. (2021). Metabolomics of potential contenders conferring antioxidant property to varied polar and non-polar solvent extracts of Edgaria darjeelingensis CB Clarke. *Bulletin of the National Research Centre,* 45, 48. https://doi.org/10.1186/s42269-021-00503-3

Chan, E.W.C., Wong, S.K., Tangah, J., Inoue, T. and Chan, H.T. (2020). Phenolic constituents and anticancer properties of *Morus alba* (white mulberry) leaves. *Journal of Integrative Medicine,* 18(3), 189-195. https://doi.org/10.1016/j.joim.2020.02.006

Chen, C., Mokhtar, R.A.M., Sani, M.S.A. and Noor, N.Q.I.M. (2022). The Effect of Maturity and Extraction Solvents on Bioactive Compounds and Antioxidant Activity of Mulberry (*Morus alba*) Fruits and Leaves. *Molecules,* 27(8), 2406-2426. https://doi.org/10.3390/molecules27082406

Chen, H.S., Bai, M.H., Zhang, T., Li, G.D. and Liu, M. (2015). Ellagic acid induces cell cycle arrest and apoptosis through TGF-β/Smad3 signaling pathway in human breast cancer MCF-7 cells. *International Journal of Oncology,* 46(4), 1730-1738. https://doi.org/10.3892/ijo.2015.2870

Chikara, S., Nagaprashantha, L.D., Singhal, J., Horne, D., Awasthi, S. and Singhal, S.S. (2018). Oxidative stress and dietary phytochemicals: Role in cancer chemoprevention and treatment. *Cancer Letters,* 413, 122-134. https://doi.org/10.1016/j.canlet.2017.11.002

Das, P.R., Darwish, A.G., Ismail, A., Haikal, A.M., Gajjar, P., Balasubramani, S.P., Sheikh, M.B., Tsolova, V., Soliman, K.F. and Sherif, S.M. (2022). Diversity in blueberry genotypes and developmental stages enables discrepancy in the bioactive compounds, metabolites, and cytotoxicity. *Food Chemistry,* 374, 131632. https://doi.org/10.1016/j.foodchem.2021.131632

Dat, N.T., Binh, P.T.X., Van Minh, C., Huong, H.T. and Lee, J.J. (2010). Cytotoxic prenylated flavonoids from *Morus alba*. *Fitoterapia,* 81(8), 1224-1227. https://doi.org/10.1016/j.fitote.2010.08.006

Deepa, M., Sureshkumar, T., Satheeshkumar, P.K. and Priya, S. (2013). Antioxidant rich *Morus alba* leaf extract induces apoptosis in human colon and breast cancer cells by the downregulation of nitric oxide produced by inducible nitric oxide synthase. *Nutrition and Cancer,* 65(2), 305-310. https://doi.org/10.1080/01635581.2013.748924

Deka, S.J., Gorai, S., Manna, D. and Trivedi, V. (2017). Evidence of PKC binding and translocation to explain the anticancer mechanism of chlorogenic acid in breast cancer cells. *Current Molecular Medicine,* 17(1), 79-89. https://doi.org/10.2174/1566524017666170209160619

El-Baz, F.K., Hassan, A.Z., Abd-Alla, H.I., Aly, H.F. and Mahmoud, K. (2017). Phytochemical analysis, assessment of antiproliferative and free radical scavenging activity of *Morus alba* and *Morus rubra* fruits. *Phytochemical Analysis,* 10(6), 189-199. https://doi.org/10.22159/ajpcr.2017.v10i6.18029

ElKhazendar, M., Chalak, J., El-Huneidi, W., Vinod, A., Abdel-Rahman, W.M. and Abu-Gharbieh, E. (2019). Antiproliferative and proapoptotic activities of ferulic acid in breast and liver cancer cell lines. *Tropical Journal of Pharmaceutical Research,* 18 (12), 2571-2576.

Eric, W.C.C., Phui, Y.L., Lea, N.T., Suit, Y.E., Yuen, P.T. and Zhiew, C.W. (2012). Effects of drying method and particle size on the antioxidant properties of leaves and teas of *Morus alba*, *Lagerstroemia speciosa* and *Thunbergia laurifolia*. *Chemical Industry and Chemical Engineering Quarterly,* 18(3), 465-472. https://doi.org/10.2298/CICEQ111116023C

Fathy, S.A., Singab, A.N.B., Agwa, S.A., El Hamid, D.M.A., Zahra, F.A. and El Moneim, S.M.A. (2013). The antiproliferative effect of mulberry (*Morus alba* L.) plant on hepatocarcinoma cell line HepG2. *Egyptian Journal of Medical Human Genetics* 14(4), 375-382. https://doi.org/10.1016/j.ejmhg.2013.07.001

Ganash, M.A. (2021). Anticancer potential of ascorbic acid and inorganic selenium on human breast cancer cell line MCF-7 and colon carcinoma HCT-116. *Journal of Cancer Research and Therapeutics,* 17 (1), 122-129. https://doi.org/10.4103/jcrt.JCRT_989_17

Gracin, S. and Rasmuson, Å.C. (2002). Solubility of phenylacetic acid, p-hydroxyphenylacetic acid, p-aminophenylacetic acid, p-hydroxybenzoic acid, and ibuprofen in pure solvents. *Journal of Chemical and Engineering Data,* 47(6), 1379-1383. https://doi.org/10.1021/je0255170

Gug, K. (2012). Physiological and whitening effects of Morus alba extracts. *Journal of the Chosun Natural Science*, 5(1), 46-52. https://doi.org/10.13160/ricns.2012.5.1.046

Gurina, D.L., Odintsova, E.G., Golubev, V.A., Antipova, M.L. and Petrenko, V.E. (2017). Features of solvation of phenolic acids in supercritical carbon dioxide modified by methanol and acetone. *The Journal of Supercritical Fluids,* 124, 50-56. https://doi.org/10.1016/j.supflu.2017.01.012

Hasani, N.A.H., Amin, I.M., Kamaludin, R., Rosdyd, N.M.M.N.M., Ibahim, M.J. and Kadir, S.H.S.A. (2018). P53 and Cyclin B1 Mediate Apoptotic Effects of Apigenin and Rutin in ERï¡+-Breast Cancer MCF-7 Cells. *Jurnal Teknologi,* 80(1), 133-140. https://doi.org/10.11113/jt.v80.10704

He, X., Chen, X., Ou, X., Ma, L., Xu, W. and Huang, K. (2019). Evaluation of flavonoid and polyphenol constituents in mulberry leaves using HPLC fingerprint analysis. *International Journal of Food Science and Technology,* 55(2), 526-533. https://doi.org/10.1111/ijfs.14281

He, X., Fang, J., Ruan, Y., Wang, X., Sun, Y., Wu, N., Zhao, Z., Chang, Y., Ning, N. and Guo, H. (2018). Structures, bioactivities and future prospective of polysaccharides from *Morus alba* (white mulberry): A review. *Food Chemistry,* 245, 899-910. https://doi.org/10.1016/j.foodchem.2017.11.084

Ho, Y., Suphrom, N., Daowtak, K., Potup, P., Thongsri, Y. and Usuwanthim, K. (2020). Anticancer effect of *Citrus hystrix* DC. leaf extract and its bioactive constituents citronellol and, citronellal on the triple negative breast cancer MDA-MB-231 cell line. *Pharmaceuticals,* 13(12), 476-493. https://doi.org/10.3390/ph13120476

Hyun, T.K., Kim, M.O., Lee, H., Kim, Y., Kim, E. and Kim, J.S. (2013). Evaluation of anti-oxidant and anti-cancer properties of *Dendropanax morbifera* Léveille. *Food Chemistry,* 141(3), 1947-1955. https://doi.org/10.1016/j.foodchem.2013.05.021

Izzreen, M.N.Q., Fadzelly, A.M., Hartina, M.U., Amirah, R.R. and Rozzamri, A. (2020). A cytotoxicity and sub-acute toxicity study on tea leaves cultivated in Sabah. *International Food Research Journal,* 27(5), 925-933.

Jelled, A., Hassine, R.B., Thouri, A., Flamini, G., Chahdoura, H., El Arem, A., Lamine, J.B., Haouas, Z., Cheikh, H.B. and Achour, L. (2017). Immature mulberry fruits richness of promising constituents in contrast with mature ones: A comparative study among three Tunisian species. *Industrial Crops and Products,* 95, 434-443. https://doi.org/10.1016/j.indcrop.2016.10.053

Jeszka-Skowron, M., Flaczyk, E., Kobus-Cisowska, J., Kośmider, A. and Górecka, D. (2014). Optimization the extraction process of phenolic compounds and antiradical activity of white mulberry leaves by response surface methodology. *Zywnosc Nauk Technologia Jakosc,* 1(92), 148-159. https://doi.org/10.15193/zntj/2014/92/148-159

Jóźwiak, M., Filipowska, A., Fiorino, F. and Struga, M. (2020). Anticancer activities of fatty acids and their heterocyclic derivatives. *European Journal of Pharmacology,* 871, 172937-172950. https://doi.org/10.1016/j.ejphar.2020.172937

Jung, B.S., Lee, N.K., Na, D.S., Yu, H.H. and Paik, H.D. (2016). Comparative analysis of the antioxidant and anticancer activities of chestnut inner shell extracts prepared with various solvents. *Journal of the Science of Food and Agriculture,* 96(6), 2097-2102. https://doi.org/10.1002/jsfa.7324

Kandil, A.S., Abou-Elella, F. and El Shemy, H.A. (2019). Cytotoxic profile activities of ethanolic and methanolic extracts of chicory plant (*Cichorium intybus* L.). *Journal of Radiation Research and Applied Sciences,* 12(1), 106-111. https://doi.org/10.1080/16878507.2019.1594136

Khan, M.A., Rahman, A.A., Islam, S., Khandokhar, P., Parvin, S., Islam, M.B., Hossain, M., Rashid, M., Sadik, G. and Nasrin, S. (2013). A comparative study on the antioxidant activity of methanolic extracts from different parts of *Morus alba* L. (Moraceae). *BMC Research Notes,* 6(24), 24-33. https://doi.org/10.1186/1756-0500-6-24

Kim, D.S., Kang, Y.M., Jin, W.Y., Sung, Y.Y., Choi, G. and Kim, H.K. (2014). Antioxidant activities and polyphenol content of *Morus alba* leaf extracts collected from varying regions. *Biomedical Reports,* 2(5), 675-680. https://doi.org/10.3892/br.2014.294

Lee, K.M., Oh, T.J., Kim, S.H., Kim, H.Y., Chung, H., Min, D.S., Auh, J.H., Lee, H.J., Lee, J. and Choi, H.K. (2016). Comprehensive metabolic profiles of mulberry fruit (*Morus alba* Linnaeus) according to maturation stage. *Food Science and Biotechnology,* 25(4), 1035-1041. https://doi.org/10.1007/s10068-016-0167-7

Lee, Y. and Hwang, K.T. (2017). Changes in physicochemical properties of mulberry fruits (*Morus alba* L.) during ripening. *Scientia Horticulturae,* 217, 189-196. https://doi.org/10.1016/j.scienta.2017.01.042

Li, Y., Kong, D., Fu, Y., Sussman, M.R. and Wu, H. (2020). The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiology and Biochemistry,* 148, 80-89. https://doi.org/10.1016/j.plaphy.2020.01.006

Ministry of Health Malaysia (2021). National Strategic Plan for Cancer Control Programme 2021 - 2025. Retrieved on September 14, 2021 from Ministry of Health Website: https://www.moh.gov.my/moh/resources/Penerbitan/Rujukan/NCD/Kanser/National_Strategic_Plan_for_Cancer_Control_Programme_2021-2025.pdf.

Natić, M.M., Dabić, D.Č., Papetti, A., Akšić, M.M.F., Ognjanov, V., Ljubojević, M. and Tešić, Ž.L. (2015). Analysis and characterisation of phytochemicals in mulberry (*Morus alba* L.) fruits grown in Vojvodina, North Serbia. *Food Chemistry,* 171, 128-136. https://doi.org/10.1016/j.foodchem.2014.08.101

Nemdili, L., Guedjali, R., Habchi, S., Mameri, F., Koutchoukali, O., Dehane, A. and Merouani, S. (2022). Ascorbic Acid Solubility and Thermodynamic Characteristics in Several Neat Solvents with Temperatures Ranging from 293 to 313 K. *International Journal of Thermophysics,* 43, 123. https://doi.org/10.1007/s10765-022-03055-4

Ngema, P.T., Matkowska, D., Naidoo, P., Hofman, T. and Ramjugernath, D. (2012). Vapor–Liquid Equilibrium Data for Binary Systems of 1-Methyl-4-(1-methylethenyl)-cyclohexene+{Ethanol, Propan-1-ol, Propan-2-ol, Butan-1-ol, Pentan-1-ol, or Hexan-1-ol} at 40 kPa. *Journal of Chemical and Engineering Data,* 57(7), 2053-2058. https://doi.org/10.1021/je300347z

Pejin, B., Kojic, V. and Bogdanovic, G. (2014). An insight into the cytotoxic activity of phytol at in vitro conditions. *Natural Product Research,* 28(22), 2053-2056. https://doi.org/10.1080/14786419.2014.921686

Pourahmad, J., Salimi, A. and Seydi, E. (2016). Role of oxygen free radicals in cancer development and treatment. In Ahmad, R. (Ed.) Free Radicals and Diseases. IntechOpen E-Book. https://doi.org/10.5772/64787

Pucci, C., Martinelli, C. and Ciofani, G. (2019). Innovative approaches for cancer treatment: current perspectives and new challenges. *Ecancermedicalscience,* 13, 961. https://doi.org/10.3332/ecancer.2019.961

Radojkovića, M., Zekovića, Z., Jokićb, S. and Vidovića, S. (2012). Determination of optimal extraction parameters of mulberry leaves using Response Surface Methodology (RSM). *Romanian Biotechnological Letters,* 17(3), 7297-7310.

Ríos-Arrabal, S., Artacho-Cordón, F., León, J., Román-Marinetto, E., del Mar Salinas-Asensio, M., Calvente, I. and Núñez, M.I. (2013). Involvement of free radicals in breast cancer. *Springerplus,* 2(1), 404-416. https://doi.org/10.1186/2193-1801-2-404

Sánchez-Salcedo, E.M., Mena, P., García-Viguera, C., Hernández, F. and Martínez, J.J. (2015a). (Poly) phenolic compounds and antioxidant activity of white (*Morus alba*) and black (*Morus nigra*) mulberry leaves: Their potential for new products rich in phytochemicals. *Journal of Functional Foods,* 18, 1039-1046. https://doi.org/10.1016/j.jff.2015.03.053

Sánchez-Salcedo, E.M., Mena, P., García-Viguera, C., Martínez, J.J. and Hernández, F. (2015b). Phytochemical evaluation of white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry fruits, a starting point for the assessment of their beneficial properties. *Journal of Functional Foods,* 12, 399-408. https://doi.org/10.1016/j.jff.2014.12.010

Singh, S., Sharma, B., Kanwar, S.S. and Kumar, A. (2016). Lead phytochemicals for anticancer drug development. *Frontiers in Plant Science,* 7, 1667. https://doi.org/10.3389/fpls.2016.01667

Sun, J. (2007). D-Limonene: safety and clinical applications. *Alternative Medicine Review,* 12(3), 259-264.

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A. and Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians,* 71(3), 209-249. https://doi.org/10.3322/caac.21660

Tan, J.J.Y., Lim, Y.Y., Siow, L.F. and Tan, J.B.L. (2015). Effects of drying on polyphenol oxidase and antioxidant activity of *Morus alba* leaves. *Journal of Food Processing and Preservation* 39(6), 2811-2819. https://doi.org/10.1111/jfpp.12532

Themelis, T., Boschetti, S., Gelli, M. and Gatti, R. (2018). Simple Determination of 1-Deoxynojirimycin in a New Dietary Supplement by Liquid Chromatography. *Chromatographia,* 81(11), 1495-1501. https://doi.org/10.1007/s10337-018-3609-y

Wong, W.J., Zainudin, S.P., Koo, Y.H., Ho, K.Y., Lee, Z.J. and Wong, K.H. (2021). Young breast cancer and the influence of ethnicity in Sandakan: A 3-year retrospective cross-sectional study. *Asia-Pacific Journal of Clinical Oncology,* 18(5), e227-e234. https://doi.org/10.1111/ajco.13596

Wulandari, Y.R.E., Prasasty, V.D., Rio, A. and Geniola, C. (2019). Determination of 1-Deoxynojirimycin Content and Phytochemical Profiles from Young and Mature Mulberry Leaves of *Morus* spp. *OnLine Journal of Biological Sciences,* 19(2), 124-131. https://doi.org/10.3844/ojbsci.2019.124.131

Yamamoto, K., Sakamoto, Y., Mizowaki, Y., Iwagaki, Y., Kimura, T., Nakagawa, K., Miyazawa, T. and Tsuduki, T. (2017). Intake of mulberry 1-deoxynojirimycin prevents colorectal cancer in mice. *Journal of Clinical Biochemistry and Nutrition,* 61 (1), 47-52. https://doi.org/10.3164/jcbn.16-94

Younus, I., Fatima, A., Ali, S.M., Usmani, S., Begum, Z., Badar, S. and Asghar, R. (2016). A review of ethnobotany, phytochemistry, antiviral and cytotoxic/anticancer potential of *Morus alba* linn. *International Journal of Advanced Research,* 1(2), 84-96.

Zhang, R., Zhang, Y., Xin, X., Huang, G., Zhang, N., Zeng, Q., Tang, L., Attaribo, T., Lee, K.S. and Jin, B.R. (2021). Dual-Targeting Antiproliferation Hybrids Derived from 1-Deoxynojirimycin and Kaempferol Induce MCF-7 Cell Apoptosis through the Mitochondria-Mediated Pathway. *Journal of Natural Products,* 84(5), 1534-1543. https://doi.org/10.1021/acs.jnatprod.1c00014

