Original Research Article

Antioxidant Activities and Cytotoxicity of Mulberry Mistletoe (*Loranthus Parasiticus* Merr) Leaves Extracts

ABSTRACT

Herein, we investigated the effect of solvents on phytochemical components, antioxidant activities, and cytotoxicity of *Mulberry mistletoe* leaves. An FT-IR method performed to identify the essential functional groups of crude powder. Total phenolic compounds (TPC), the ascorbic acid content (AA), and total flavonoids content (TFC) were measured. Further, *in vitro* antioxidant activities were performed using different assays including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity, ferrous ion-chelating activities, reducing power, total antioxidant capacity (TAC). The cytotoxicity of the extracts was tested using MDCK cells line model. The results showed that different solvents showed a significant difference in phytochemical contents and its antioxidant activities as well as cytotoxicity. We found that pure water was remarkable higher in phytochemical values and greater antioxidant activities than pure ethanol or the water-ethanol system. In this sense, pure water may thus be considered a suitable solvent based on their acceptability for human consumption without toxic, cheap and more environmentally friendly.

Running head: Antioxidant activities and cytotoxicity of mulberry mistletoe leaves

***Keywords*:** *Antioxidant activities, Bioactive compounds, Cytotoxicity, Mulberry mistletoe, Phytochemicals.*

INTRODUCTION

Phytochemicals, especially polyphenol compounds are secondary metabolites, which have potent antioxidant activity *in vitro* due to their high reactivity as hydrogen or electron donors and they are a capability in chelating metal ions, scavenging free radicals [1,2]. Several authors have mentioned that phenolic compounds from different sources are highly associated with the health benefits with a sequence of biological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-carcinogenic, anti-mutagenic, anti-thrombotic, cardioprotective and vasodilatory effects [1,3,4]. Also, solvents and methods for extracting are crucially important to isolate bioactive compounds as well as maintain their biological properties. Therefore, the exploitation and utilization new sources of natural phenolic compounds and development a new extracting technique became one of the crucial concerns not only for pharmaceutical applications and the food industry but also for the others.

The genus *Mulberry mistletoe* (*Loranthus)* belongs to *Loranthaceae* family and is found in some Asian countries such as Vietnam, China and Japan [5]. *Mulberry mistletoe* was also known as Tầm gửi cây dâu (Vietnamese), Sang Ji Sheng (in Chinese), benalu teh (in Malay) and basokisei (in Japanese). It has also believed important herbal medicine for anticancer in many countries during the past few decades [5]. Numerous studies have evaluated the phytochemicals extracted from different parts (leaf, bark, and stem) of this plant and its antioxidant capacity, neuro-protective, anticancer and antibacterial activities [6,7,8]. However, most of the studies focused on determination of total phenolic content and antioxidant activities from this plant, the reports on the effects of various solvents on phytochemical contents and its *in vitro* antioxidants are limited. Therefore, the present

study aimed to evaluate the effect of different solvent on the phytochemical components of *Mulberry mistletoe* leaves, in relation the *in vitro* antioxidant and as well as the cytotoxicity.

MATERIALS AND METHODS

Preparation of *Mulberry mistletoe* leaves extracts

Fresh *Mulberry mistletoe* leaves were purchased from a local market in Daklak, a province of the Central Highland of Vietnam. The leaves were cleaned using distilled water to remove the foreign matters and then were oven-dried at 50^{o}C until the weight stopped fluctuating for about 72 h. Next, they were powdered using a grinder (Hanil Ultra-Power Mixer 3.2L-650W, Korea) and then were sieved by using a testing sieve 200 µm to obtain a powder for the experiment (Chung Gye Sang Gong Sa, Seoul, Korea). The powder was extracted using 3 different solvents (pure water: WS; ET50: water + ethanol, 50:50; ET100: pure ethanol) with a magnetic stirrer (vigorously gentle stirring) for 3 h at room temperature (RT, approximate at 22^{o}C). The mixture was then centrifuged at 3000 rpm for 3 min (VS-5000N, Vision scientific Co. Ltd., Korea). Once centrifuged, the mixtures of solid-liquid were filtered using a Whatman #1 filter paper. The alcohol was removed from the extracted solution using a rotary evaporator (R-100 rotary evaporator, Buchi, Switzerland) if required. Thereafter, the extract was at -70^{o}C prior to lyophilizing at -55^{o}C (Ilshin freeze dryer, Korea) until completely dry for about 3 days. All samples were then kept in a refrigerator at 2^{o}C before analysis the phytochemical compounds, antioxidant activities and its cytotoxicity measurement.

Analytical methods on *Mulberry mistletoe* leaves

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Ferrozine, Folin-ciocalteu's phenol reagent, Quercetin and Potassium acetate were purchased from Sigma-Aldrich; Ascorbic acid, gallic acid, sodium carbonate, ammonium molybdate, sulfuric acid, trichloroacetic acid, ferric chloride and ferrous chloride were from Junsei (Japan); sodium phosphate (Yakuri, Japan), potassium ferricyanide (Avocado Research Chemical, UK), and aluminium nitrate from Samchun (Korea). All the chemicals including solvents were of analytical grade.

Fourier transform infrared spectrophotometer (FT-IR)

The dried powdered of *Mulberry mistletoe* leaf after grinding and sieving were subjected to FT-IR analysis using a Frontier FT-IR/FIR spectrometer (PerkinElmer FTIR/NIR 400, USA). The FT-IR spectra obtained in attenuated total reflectance (ATR) mode in the wavelength ranging from 4000 to 400 cm^{-1} and the peaks were analyzed using the PerkinElmer Spectrum Version 10.03.05.

Total phenolic compounds

The total phenolic compounds was measured as gallic acid equivalents using the Folin–Ciocalteau's phenol reagent (FC reagent) according to the method of Lin and Tang (2007) [9] with a slight modification. The extracted solution of each sample (100 µL) was mixed with 2.8 mL of deionized water, followed by the addition of 2 mL of 2.0% (w/v) Na_2CO_3. Finally, 100 µL of 50% (v/v) Folin–Ciocalteu reagent in deionized water was added, then mixed well on a vortex vibrator for 30 sec and incubated in the dark at room temperature for 30 min before the absorbance was measured at 750 nm using a UV spectrophotometer (UV 1601, Shimadzu, Australia) against the blank with same preparation by only replacing 100 µL of Folin–Ciocalteau's phenol reagent with the same volume of pure water. Gallic

acid (0-500 µg/mL) was used as a standard solution for the calibration curve. The results were expressed in milligrams of gallic acid equivalent per g of dry leaves (mg GAE g^{-1}).

Ascorbic acid content

The ascorbic content was determined using the method as described by Park *et al.* (2008) [10]. Briefly, 0.4 mL of each extract was added to the Falcon tubes with 1.6 mL of TCA 10% (100 mg/mL) and well mixing. Then, the tubes were centrifuged at 3000 rpm for 5 min. Once centrifuged, 0.5 mL of the supernatant was transferred to new tubes and mixed with 1.5 mL of pure water. Finally, 0.2 mL of Folin–Ciocalteau's phenol reagent (10% in water, v/v) was added. The mixture was incubated for 10 min at RT, and then the absorbance was measured at 760 nm by UV–vis spectrophotometer (UV 1601) against the blank with same preparation by only replacing 0.2 mL of Folin–Ciocalteau's phenol reagent with the same volume of pure water. Ascorbic acid standard solution (0-500 µg/mL) was similarly prepared and measured. The ascorbic acid equivalence of the extracts calculated based on the standard solution curve.

Total flavonoids content

The total flavonoids content was determined according to the aluminum chloride colorimetric method as described by Lin and Tang (2007) [9] with a slight modification. Firstly, 0.5 mL of each extract was mixed with 100 µl of the 10% (w/v) aluminum nitrate solution and sharked up, next 100 µl of the 1 M potassium acetate was added. The mixture was further diluted with ethanol 80% (4.3 mL) up to 5 mL. The mixture was then left in the dark and allowed to react for 40 min at RT. The absorbance of the samples was measured at 415 nm using a UV–vis spectrophotometer (UV 1601) against the blank with same preparation by only replacing 100 µl of the 10% (w/v) aluminum nitrate with the same

volume of pure water. Quercetin standard solution (0-500 µg/mL) was similarly prepared and measured. The total flavonoids content was calculated and expressed as mg quercetin equivalent per g of dried leaves powder (mg QE g^{-1}).

DPPH radical scavenging assay

DPPH radical-scavenging ability was measured using the method of Huang *et al*. (2006) [11] with some modification. Different concentrations (2000, 1000, 750, 500, 250, 125, 62.5 and 31.2 µg/mL) of the extracts were prepared. Then, one mL of each extract was mixed with one mL of freshly made DPPH solution (0.2 mM in pure methanol). The mixture was shaken and incubated in the dark for 60 min at RT. The appropriate volume of the same solvent used for the sample was used instead of the samples in the control group, and quercetin (Sigma) was used as a positive reference. The absorbance then was measured at 517 nm. DPPH radical scavenging ability was calculated using the following equation:

DPPH radical-scavenging activity (%) = $[(ABS_{ctl} - ABS_{spl}) / ABS_{ctl}] \times 100$

Where: ABS_{ctl} is the absorbance value of the control group, and ABS_{spl} is the absorbance of the samples. The nonlinear concentration−inhibition response was plotted, and 50% inhibition concentration (IC_{50}) was calculated.

Ferrous ion-chelating ability

The ferrous ion-chelating ability of the leaf extracts was evaluated by measuring the inhibition of the formation of a Fe^{2+}ferrozine complex using the method described by Le *et al.* (2007) [12] with a slight modification. 0.5 mL of leaves extract of different solvents, 0.1 mL 0.6 mM (in pure water) ferrous chloride ($FeCl_2$), and 0.9 mL methanol were combined. The mixture was shaken well and allowed to react for 5 min at room temperature. After the reaction, ferrozine (0.1 mL, 5 mM in methanol) was added and kept

further 10 min for reaction at RT. The absorbance was then measured at 562 nm. The chelating ability was calculated as a percentage via the following equation:

Chelating ability (%) = $[(1 - ABS_{spl}/ABS_{ctl}] \times 100$

Where: ABS_{ctl} is the absorbance value of the control group, and ABS_{spl} is the absorbance of the samples.

Reducing power

The reducing power was measured via the method described by Le *et al.* (2007) [12]. Each mixture contained 2.0 mL of leaf extracts, 2.0 mL of sodium phosphate buffer (0.2 M, pH 6.6), and 2.0 mL of potassium ferricyanide (10 mg/mL). The mixture was incubated in a water-bath for 20 min at 50^{o}C. Then, after cooling to RT, 2.0 mL of trichloroacetic acid 10% (100 mg/mL) was added to stop the reaction and then was centrifuged for 10 min at 2000 rpm. Once centrifuged, the upper layer (2.0 mL) was mixed with 2.0 mL of distilled-water and 0.4 mL of ferric chloride (1.0 mg/mL). The absorbance at 700 nm was measured and high values regarding high reducing power.

Total antioxidant capacity by phosphomolybdenum reagent

The total antioxidant capacity of the leaf extracts was determined using the method of Prieto *et al*. (1999) [13] with a slight modification. Briefly, 100 µL of leaf extracts were mixed with one mL of the reagent solution (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulfuric acid). Then, they were incubated in a water-bath at 95^{o}C for 90 min. After the samples cooled to RT, the absorbance of the samples was measured at 695 nm. The ascorbic acid solution was prepared (0-1000 µg/mL) and used as a positive standard. The total antioxidant capacity of the samples was expressed as milligrams of ascorbic acid equivalent per gram of dry weight (mg AAE g^{-1}).

Cells culture and cytotoxicity assay

The cytotoxicity of leave extracts was evaluated using Madin−Darby canine kidney (MDCK) cells. Cell viability was measured by the Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Kumamoto, Japan) method. MDCK cells were seeded in 96-wells plates at a density of 2×10^4 cells per well and incubated for 24 h in Dulbecco's modified Eagle's medium (DMEM) with the addition of 10% heated FBS and antibiotics (streptomycin 100 mg/mL and penicillin 100 U/mL, Sigma-Aldrich, St. Louis, MO, USA). After the cell monolayer formation, cells were washed with PBS. The extracted compounds were dissolved in DMSO to 10 mg/mL, and serial twofold dilutions with DMEM to obtain the final concentration of 1000, 500, 250, 125, 62.5, and 31.2 µg/mL. The dilutions of the extracts were used to treat the MDCK cells and incubated for 48 h at 37^oC, 5% CO_2. Then CCK-8 kit reagent was added and after the incubation time (1 h, at 37^oC, and 5% CO_2), the absorbance was measured at 450 nm using a microplate reader (Synergy, Bio-Tek, VT, USA), and cytotoxicity was calculated as a percentage via the following equation:

Cell viability (%) = [A-B] / [C-B] x 100

Where A, B, and C are the absorbance of the test sample (extracts treated cells), background (medium/extracts without cells), and negative control (control medium with cells), respectively. Nonlinear concentration−response curves were plotted, and half-maximal cytotoxic concentration (CC_{50}) was calculated.

Statistical analysis

All experiments were carried out in triplicate and data were analyzed using one-way analysis of variance (ANOVA). The significant differences were assessed by the Duncan

test at *p*-value < 0.05 using Statistical Package for the Social Sciences software for Windows (SPSS IBM version 20.0). Results were presented as the mean ± standard deviation (SD). Figure and IC_{50}/CC_{50} values were performed using Graph-Pad Prism software version 5.01 (Graph-Pad Software Inc., USA).

RESULTS AND DISCUSSION

Effects of solvents on phytochemical components of *Mulberry mistletoe* leaf

FT-IR analysis

FT-IR analysis was carried out to identify the chemical structure of individual antioxidant components from *Mulberry mistletoe* leaf. As shown in Fig. 1, six major peaks with different transmittance and their functional groups from the leaf powder were detected including 3266.83 cm^{-1} (-OH stretching vibration), 2918.59 cm^{-1}(-CH stretching vibration), 1618.94 cm^{-1}(-NH stretching vibration), 1316.82 cm^{-1}, 1239.48 cm^{-1} (-CH_2 stretching) and 1026.92 cm^{-1} (C-C, C-OH, -CH ring and side group vibrations). In fact, FT-IR analysis confirmed that *Mulberry mistletoe* leaf powder contains phenol, alcohol, alkane, alkyne, aromatics, hydrocarbons and amines. Our results were similar to the finding of Subashini *et al*. (2015) who reported that *Gymnema sylvestre* leaves contained alcohols, phenols, alkanes, alkynes, alkyl halides aldehydes, carboxy acids aromatics, and aromatic amines [14]. Earlier, Sangeetha *et al*. (2014) stated that the presence of aliphatic, aromatic amines and alkenes in *Gymnema sylvestre* might contribute to the antioxidant activity [15]. In addition, in a previous study of Jabamalairaj *et al*. (2015) about the *Citrus grandis* (L.) leaves indicated that the presence of functional groups such as alcohol, alkane, amines, aromatics, aldehydes, phenols, esters and nitro compounds correlated with antimicrobial

209 activity [16]. Thus, these compounds from extracts of *Mulberry mistletoe* leaves may
210 function as antioxidant and antimicrobial agent.

211 *Total phenolic compound (TPC)*

212 The amounts of TPC of three different solvents were shown in Fig. 2A. TPC of *Mulberry*
213 *mistletoe* leaves by different solvents ranged from 19.25 to 63.18 mg GAE/g, which had
214 relatively higher amount when compared to other plant species. Zhou and Yu (2006)
215 reported that the levels of TPC of 38 commonly consumed vegetable samples in Colorado
216 were ranged from 2.9 to 18.8 mg GAE g^{-1} of dry matter [17]. The results of this study
217 indicate that the quantity of TPC was significantly different among solvents. In fact, WS
218 extract contained the highest TPC compounds at 63.18 mg GAE g^{-1} whereas, ET50 was
219 lower at 40.07 mg GAE g^{-1} while ET100 was the lowest at 19.25 mg GAE g^{-1}. Results did
220 not show the similarity with the earlier observations [18], which indicated that pure water
221 was the least effective solvent for the extraction of total phenolic compounds from plants
222 in comparison with the other solvents. However, our finding was in agreement with Vuong
223 *et al.* (2013), who reported that water extract contains the highest polyphenols from papaya
224 leaf and black tea compared to pure acetone, ethanol, and methanol [19]. The results could
225 be explained that plants contained a diverse group of secondary metabolites such as
226 phenolic acids, flavonoids, etc., which have different polarity. Therefore, the type and
227 quantity of phenolic compounds being dissolved in the different solvents also differ.
228 Moreover, several authors have stated that by the change of solvent polarity, conditions
229 extraction (vapor pressure, ratio, time extraction, and temperature) and viscosity have a
230 positive effect to the extractability [18,19,20]. In general, a direct relationship was found
231 between the amount of extracted phenolic compounds and the solvent polarity. As solvent

polarity changed, the yield extractions of TPC were different accordingly. Similarly, Thoo *et al.* (2010) and others also reported that lower ethanol concentration contained a higher proportion of total phenolic compounds [20].

The total flavonoids content (TFC)

It had been stated by earlier observation, which reported that the potential antioxidant activity of flavonoids is related to the chemical structures, which contain multiple hydroxyl functions substitutions with the o-diphenolic group, a 2–3 double bond in conjugation with the 4-oxo function and hydroxyl groups in positions 3 and 5 [18]. TFC from the leaf by different extracts is shown in Fig. 2B, the values were varied from 3.02 to 4.97 mg QE g^{-1}, which the highest in WS, while no significant difference between ET50 and ET100, and those values were lower than WS. Thus, pure water was more appropriate for extraction of TFC than those of ethanol from this leaves.

The content of ascorbic acid (AA)

AA from the extracts of *Mulberry mistletoe* leaves is shown in Fig. 2C, in fact, WS had the highest content (13.07 mg AA g^{-1}) followed by ET50 (8.09 mg AA g^{-1}) and ET100 was the lowest at the level of 1.68 mg AA g^{-1} (p<0.05). WS was more efficient means for extraction ascorbic acid content from the leaf than a water-ethanol system. It could be explained that because the ascorbic acid is water-soluble complex, hence more AA was contained in WS extract. Additionally, based on the result, it can be indicated that the ascorbic content in the extracts correlated with the total phenolic compounds, being highest in WS and lower in a mixture of ethanol at 50% or pure ethanol.

***In vitro* antioxidant activities**

Many previous studies had shown that phytochemicals from plants and vegetables are believed to provide potential antioxidant. Also, it is known that the bioactive compounds such as phenolics, flavonoids produce a broad spectrum of unique biological effects. Still, much interesting is remaining to find out new sources and new methods to assess and isolate antioxidant from natural for a variety of applications. Earlier, it has been opined that the difference in the structure of phenolic components, as well as the methodology of the antioxidant assay, may cause different results in the assessment of antioxidant ability [21]. Therefore, for the antioxidant activities evaluation from different plant extracts must be measured by using numerous *in vitro* assays for different mechanisms to get relevant values. In this work, different antioxidant tests were carried out including DPPH ability, reducing power, ferrous ion chelating and total antioxidant capacity.

DPPH radical scavenging ability

The DPPH ability of three different extracts from *Mulberry mistletoe* leaves by percent inhibition was presented in Fig. 3A and 3E. The result shows *Mulberry mistletoe* leaves extracts had a potent free radical scavenging activity as compared to quercetin. Since DPPH radical scavenging ability is one of the most commonly used methods to evaluate the antioxidant activity of various sources, herein we investigated DPPH ability of leave extracts of different concentration. As shown in Fig. 3E, the DPPH values ranging from 20.96 to 77.67% for WS and 5.42 to 74.75% for ET50, whereas DPPH of quercetin was from 59.79 to 91.46% (at the concentration ranging from 31.2 to 2000 μg/mL). Thus, WS extract exhibited a greater ability than ET50 at certain concentrations. Together with DPPH radical scavenging ability, the IC_{50} value as shown in Fig. 3F, WS extract (IC_{50}=345.3 μg/mL) was substantially lower than ET50 (IC_{50}=630.7 μg/mL), while

277 quercetin showed the lowest value at 27.14 µg/mL. Our result was different with previous
278 findings, which stated that all extracts obtained by using a pure and aqueous organic
279 solvent gave stronger DPPH ability than that of the water extract [18,22]. Changing in
280 solvent polarity alters its ability to dissolve a selected group of antioxidant compounds and
281 influences the antioxidant activity estimation. Thus, it can be indicated that using solvents
282 with higher in polarity were considerably more efficient for extracting of radical
283 scavenging compounds from this plant.

284 *Ferrous ion-chelating ability (FIC)*

285 The results of the FIC assay were plotted as percentage chelating effect by various
286 solvent extracts are shown in Fig. 3B. The FIC of various extracts from *Mulberry mistletoe*
287 leaves followed the order ET80 ≈ pure water > ET50. In fact, pure water extract of
288 *Mulberry mistletoe* leaves showed strong FIC ability (100.58%), was similar in value to
289 ET100 (101.56%). Interestingly, the result showed that ET50 was lower chelating activity
290 than pure water and ET100 (p<0.05). Because might be due to the complex composition of
291 *Mulberry mistletoe* leaves, which contained a various group of antioxidant potential with
292 differing in polarity and various mechanisms with a higher proportion of hydrophilic
293 compounds. In this case, it can be observed that pure water was more favorable in the
294 extracting of the ion chelate compounds in this leaf as compared to the other solvents. Our
295 result was similar to the study of Yeşiloğlu and Şit (2012), whose showed that the
296 percentages of ion chelating capacity of water extract were higher than those of ethanol or
297 acetone [23]. It suggested that pure water might be a good solvent for extraction the ion
298 chelate components from *Mulberry mistletoe* leaf.

299 *Reducing power ability (RDP)*

The RDP of various extracts is presented in Fig. 3C, which showed that the reducing power ability was dependent on the solvents used (p<0.05). In fact, the higher RDP was obtained in pure water extract and aqueous solvents at 50% as compared to pure ethanol. However, our results were different from the finding of Anwar and Przybylski (2012) [24], who reported that RDP was highest in pure methanol extract, which higher in values than 80% ethanol and 80% methanol extract. The difference between these results could be explained that may be due to the variety of the plant materials was various mechanisms might contribute to oxidative processes.

Total antioxidant capacity

The total antioxidant capacity (TAC) of different solvent extracts was measured and expressed as mg ascorbic acid equivalents (AAE) g^{-1} dry leaves. The result was presented in Fig. 3D, which shows that pure water displayed the highest antioxidant capacity with TAC value of 27.13 mg AAE g^{-1} followed by ET50 at 22.52 mg and the lowest for ET100 at 17.43 mg (p<0.05). These significant variations indicated that change in polarity, the vapor pressure of solvent might significantly influence the antioxidant capacity. Several previous studies have measured the effect of different solvents on antioxidant activity using different methods, and they reported the results differently [18,19,22,23,24,25]. Our study showed that pure water had the strong total antioxidant capacity. It could be explained that almost antioxidant compounds in this leaf were mostly water-soluble components (hydrophilic groups). On the other hand, this result confirmed that there is a good correlation between TPC and TAC. Therefore, based on the result, it could be revealed that phenolic compounds of the *Mulberry mistletoe* leave extracts would have the utmost importance of the total antioxidant capacity.

Correlation between phytochemicals and antioxidants

Since it was important to know the correlation between TPC and TAC, the Pearson's correlation coefficient analysis was carried out (Table 1). The results obtained from correlation between phytochemicals (TPC, TFC, AA) and antioxidants showed that total phenolic compound (TPC) and total antioxidant capacity (TAC) are highly correlation (r=0.998, $p<0.05$). It suggested that TPC are the dominant contributor to the antioxidant activity of the leaf extract. This result was in agreement with Kchaou *et al.* (2013), which reported that the good correlation between total phenols analysis and antioxidant assays [3]. The results showed that reducing power (RDP) was linearly positive correlated to DPPH (r = 0.997, $p<0.05$), however in case of FIC, it was a weak correlation. Moreover, our result showed that FIC ability and phytochemicals (TPC, TFC, and AA) was reversible or no relationship between them was observed with r values -0.136, 0.426, and -0.238, respectively (Table 1). Therefore, it can be indicated that these types of compounds (TPC, TFC, and AA) do not make a significant contribution to the FIC ability and may be due to their complex composition from this leaf, which contained a broad of secondary metabolite compounds with differing in polarity and various mechanisms.

Cytotoxicity of *Mulberry mistletoe* extracts

As a result, is shown in Fig. 4A-B, *Mulberry mistletoe* extracts changed efficiently the viability of MDCK cells at the concentrations in the range of 31.2–2000 µg/mL. Interestingly, the results showed that water extract had no cytotoxic effect on MDCK cells at the concentration below 500 µg/mL, whereas ET50 extract was toxic at the dose higher than 100 µg/mL. The CC_{50} value of WS (CC_{50} = 1604 µg/mL) was much higher than ET50 extract (471.8 µg/mL), which indicates that WS extract from *Mulberry mistletoe* was lower

cytotoxicity than ET50 extract at certain concentrations. The different in the cytotoxic dose of leave extracts may in part be due to the specific compound of phytochemical characteristics of various solvents, which showed in results mentioned above.

CONCLUSION

The results showed a possible influence of extracting solvents on the phytochemical components and antioxidant activities from *Mulberry mistletoe* leaves. Pure water or ethanol at low concentration might obtain the higher total phenolic compounds, flavonoids content, and maintained antioxidant activities than those of pure ethanol. Thus, the current result demonstrated that pure water could be considered an excellent solvent to extract health beneficial bioactive compounds based on their acceptability for human consumption without toxic, cheap and more environmentally friendly. However, further investigation on the role played by specific molecules or individual phenolics from *Mulberry mistletoe* leaves on the potential biological activities such as antidiabetic, antibacterial, antiviral and anticancer in both of *in vitro* and *in vivo* would be required.

Conflict of Interests

The authors declare no conflict of interests regarding the publication of this paper.

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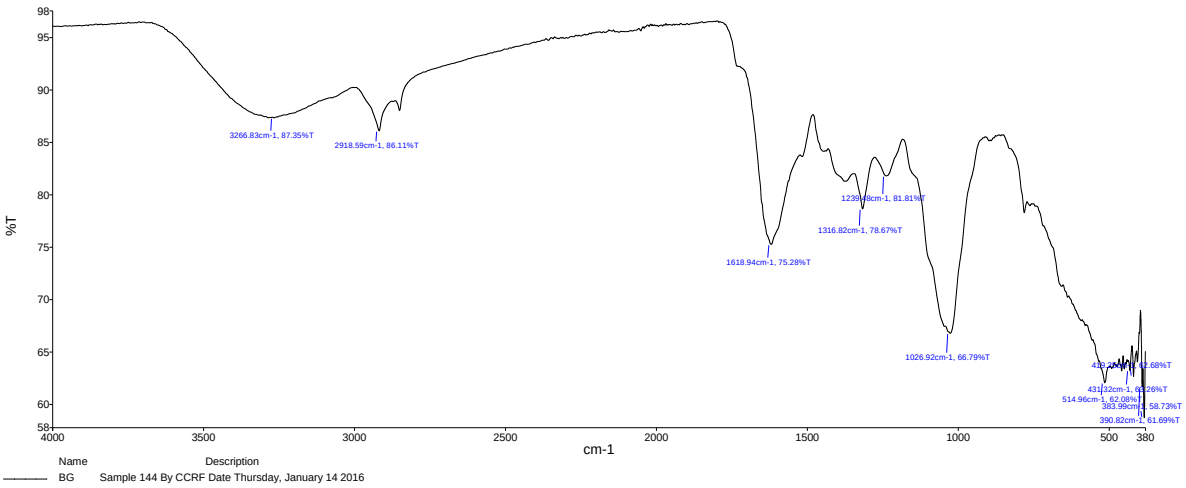
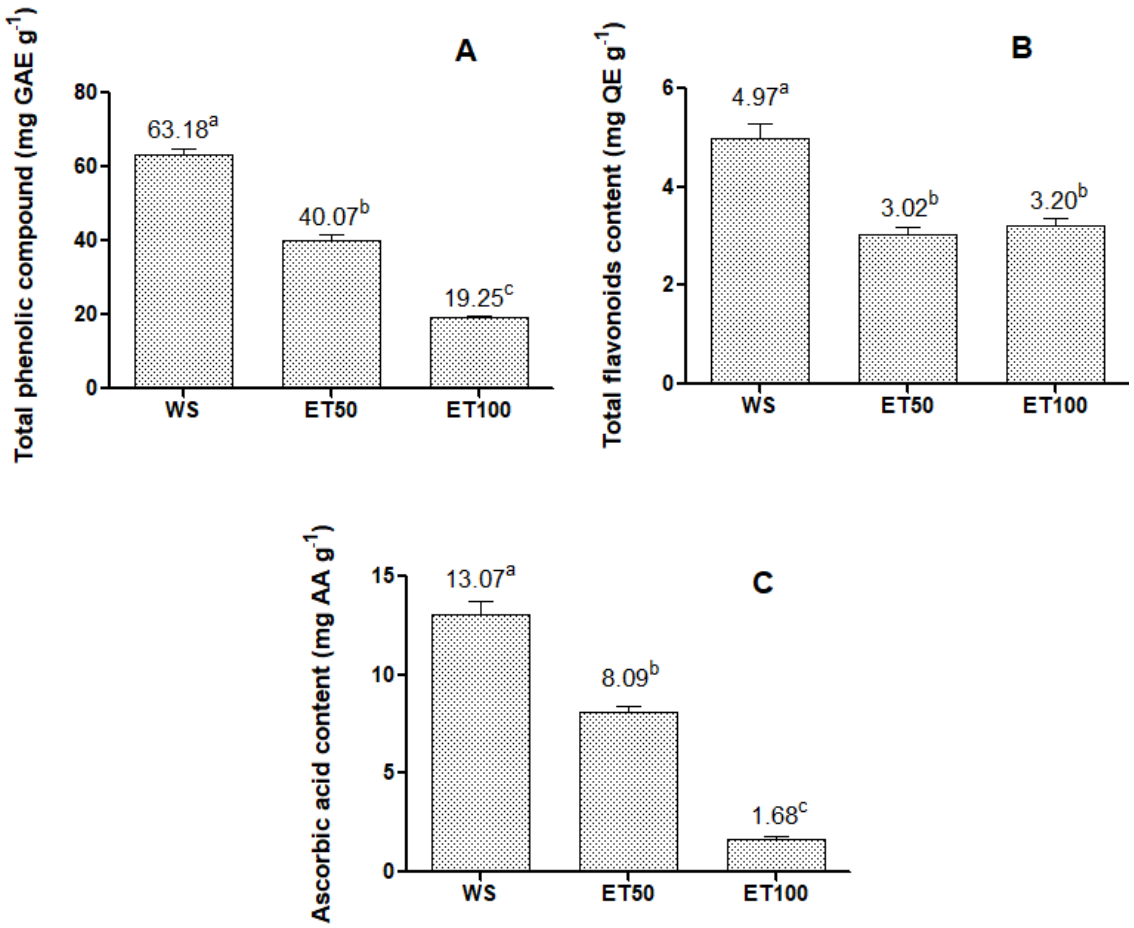


Fig. 1. FT-IR analysis of *Mulberry mistletoe* leaves crude powder. The spectrum was analyzed in the Spotlight 400 FT-IR, Perkin Elmer systems at the wavelength ranging from 4000 to 400 cm^{-1} and the peaks were analyzed using the Perkin Elmer Spectrum Version 10.03.05.



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462 **Fig. 2. The phytochemical equivalence of *Mulberry mistletoe* leaves as affected by**

463 **different solvents.** Total phenolic content (A), Total flavonoids content (B), and Ascorbic acid content

464 (C). Values (Mean ± SD of triplicate) with different superscript letters ($^{a\text{-}c}$) above bars indicate significant

465 difference from one another at $p < 0.05$ (Duncan's test). GAE: gallic acid equivalent; QE: Quercetin

466 equivalent; AA: Ascorbic acid equivalent.

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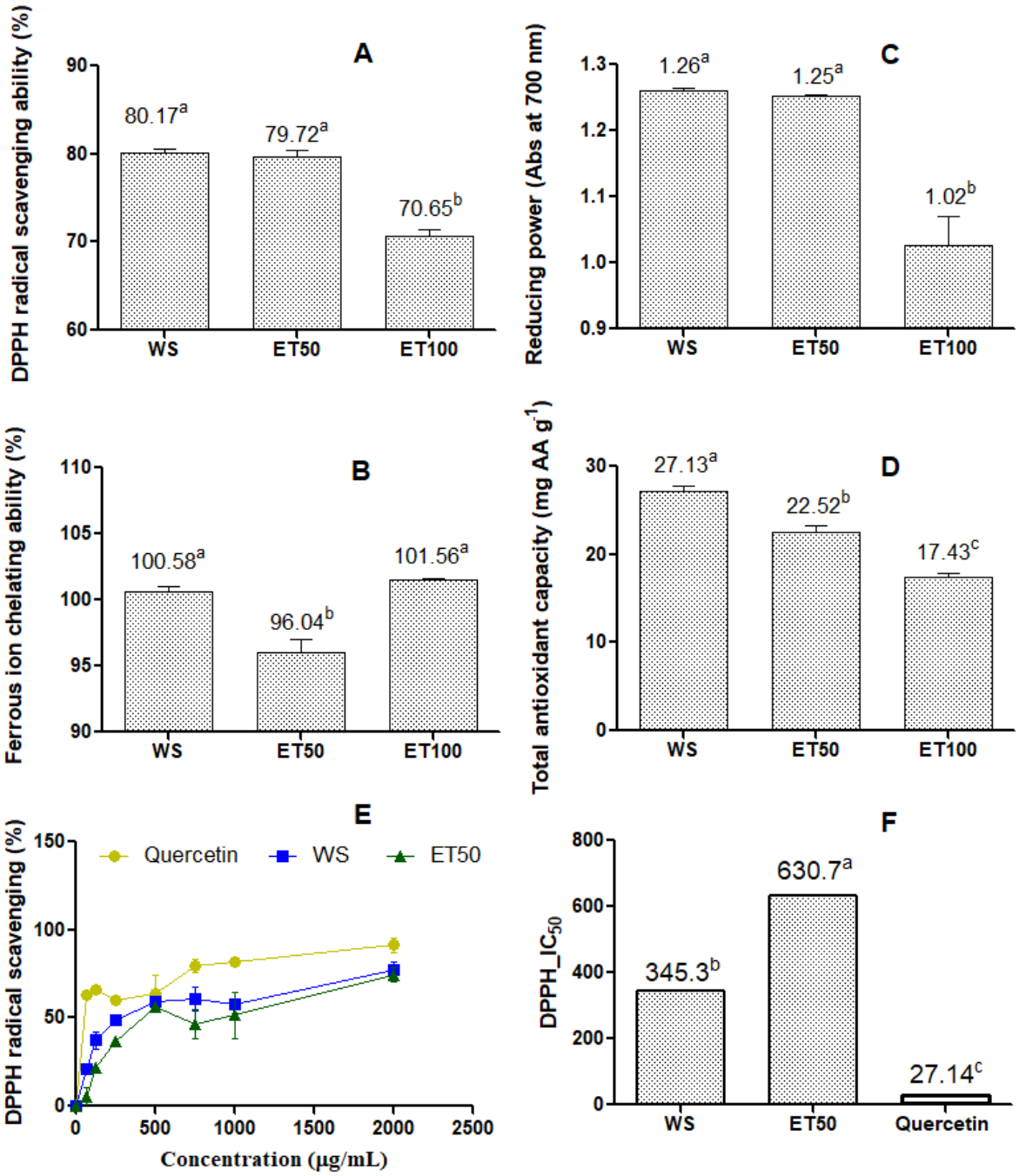

Fig. 3. Antioxidant activities of leave extracts. DPPH radical scavenging ability (A), Ferrous ion-chelating ability (B), Reducing power ability (C), Total antioxidant capacity (D), DPPH radical scavenging ability at different concentration (E) and together with its IC_{50} values (F). Values (Mean ± SD of triplicate) with different superscript letters ($^{a-c}$) above bars indicate significant difference from one another at $p < 0.05$ (Duncan's test).

474 **Table 1.** Relationship between antioxidant assays and presence of phytochemical
475 compounds from *Mulberry mistletoe* leaf described by correlation coefficient

Variables	TPC	TFC	AA	DPPH	FIC	RDP	TAC
TPC		0.838	0.995	0.895	-0.136	0.859	**0.998***
TFC	0.838		0.777	0.507	0.426	0.441	0.804
AA	0.995	0.777		0.936	-0.238	0.907	**0.999***
DPPH	0.895	0.507	0.936		-0.564	**0.997***	0.920
FIC	-0.136	0.426	-0.238	-0.564		-0.624	-0.194
RDP	0.859	0.441	0.907	**0.997***	-0.624		0.888
TAC	**0.998***	0.804	**0.999***	0.920	-0.194	0.888	

476 (*r* values; n = 3), Pearson correlation (2-tailed)

477 *. Correlation is significant at the $p<0.05$ level

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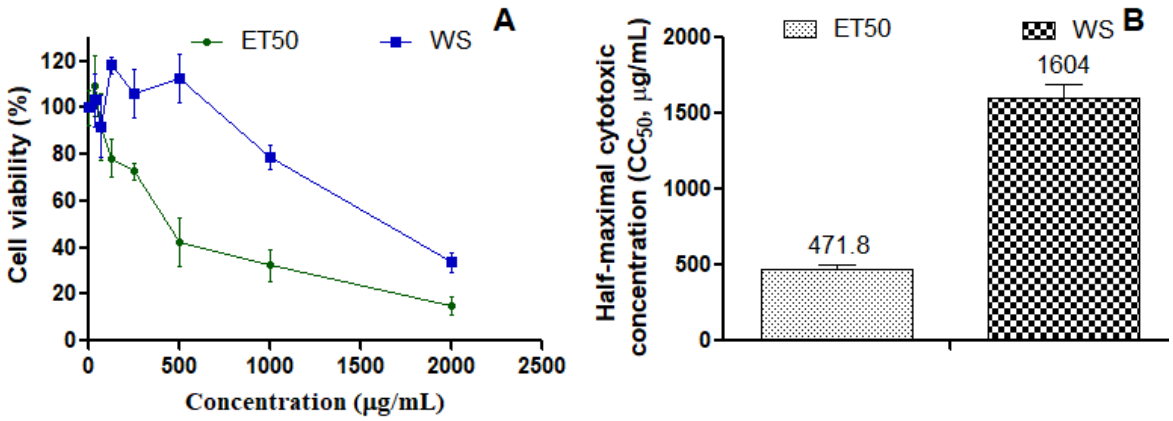

Fig. 4. Cytotoxicity of *Mulberry mistletoe* leaves extracts on MDCK cells. MDCK cells were treated with different concentration of *Mulberry mistletoe* extracts for 48 h, and CCK-8 kit was added to measure cells viability. Cell viability was expressed as a percentage of the viability of Mock (blank control). A: Cell viability (%) of different concentration of the extracts, B: half-maximal cytotoxic concentration (CC_{50}). Each value represents the mean ± SD of triplicate.