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Phytochemical Components, Antioxidant and Cytotoxic Activities of Mulberry Mistletoe (*Loranthus parasiticus* Merr) Leaves Extracts

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors NTT and TVC contributed equally to this study. Author TVC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NTT and TVC managed the analyses of the study. Author NTT managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Herein, we investigated the effects of solvents on the phytochemical components, antioxidant activities, and cytotoxicity of *Mulberry mistletoe* leaves. An FT-IR method was performed to identify the essential functional groups of crude powder. Total phenolic compounds, ascorbic acid content, and total flavonoids content were measured. Further, *in vitro* antioxidant activities were performed using different assays including 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity, ferrous ion-chelating activities, reducing power, total antioxidant capacity. The cytotoxicity of each extract was tested using the MDCK cell line. The results showed that different solvents showed a significant difference in phytochemical contents, antioxidant activities, as well as cytotoxicity. We

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found that pure water extraction had remarkably higher 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 its acceptability for human consumption without toxicity, low cost and environmental friendliness.

Keywords: Antioxidant activities; cytotoxicity; Mulberry mistletoe; phytochemicals.

ABBREVIATIONS

AA: Ascorbic acid; AAE: Ascorbic acid equivalent; ANOVA: Analysis of variance; ATR: Attenuated total reflectance; CCK-8: Cell counting kit-8; CC_{50}: Half-maximal cytotoxic concentration; DMEM: Dulbecco's modified Eagle's medium; DMSO: Dimethyl sulfoxide; DPPH: 1,1-diphenyl-2-picrylhydrazyl; EDTA: Ethylene-Diamine-Tetra-Acetic acid; ET50: Water + Ethanol (50:50, v/v); ET100: Pure ethanol; FBS: Fetal bovine serum; FC: Folin–Ciocalteau's phenol; FIC: Ferrous iron chelating; FT-IR: Fourier-transform infrared; GAE: Gallic acid equivalent; IC_{50}: 50% inhibition concentration; MDCK: Madin−Darby canine kidney; PBS: Phosphate-buffered saline; RDP: Reducing power; RT: Room temperature; TCA: Trichloroacetic acid; TFC: Total flavonoids content; TPC: Total phenolic compounds; QE: Quercetin equivalent; WS: Pure water.

1. 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, their capability in chelating metal ions, and free radical scavenging activity [1,2]. Several authors have mentioned that phenolic compounds from different sources have several 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 for isolating bioactive compounds as well as maintaining their biological properties. Therefore, the exploitation and utilization of natural phenolic compounds from new sources and the development of a new extraction technique have become crucial concerns, not only for pharmaceutical applications and the food industry, but also for other fields.

Mulberry mistletoe (*Loranthus)* belongs to the *Loranthaceae* family and is found in some Asian countries such as Vietnam, China, and Japan [5]. *Mulberry mistletoe* is also known as Tầm gửi cây dâu (Vietnamese), Sang Ji Sheng (in Chinese), benalu teh (in Malay) and basokisei (in Japanese). It has also been believed to be an important herbal medicine against cancer in many countries over the past few decades [5]. Increasing research on traditional herbal medicines and their phytoconstituents have recognized their usefulness in the treatment of various diseases. Thus, numerous studies have evaluated the phytochemicals extracted from different parts (leaves, branches, bark, and stem) of this plant and its antioxidant, neuro-protective, anti-inflammatory, anticancer and antibacterial activities [6,7,8]. However, most of the studies focused on the determination of the total phenolic contents and antioxidant activities from this plant, so reports on the effects of various solvents on phytochemical contents, and its *in vitro* antioxidant activities, are limited. Therefore, the present study aimed to evaluate the effects of different solvents on the phytochemical components of *Mulberry mistletoe* leaves, in relation to the *in vitro* antioxidant activity, as well as the cytotoxicity.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Ferrozine, Folin-ciocalteu's phenol reagent, quercetin, EDTA, 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 purchased 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.

2.2 Preparation of Mulberry Mistletoe Leaf Extracts

Fresh *Mulberry mistletoe* leaves were purchased from a local market in Daklak, a province in the Central Highlands of Vietnam. The leaves were cleaned using distilled water to remove foreign matter, and then were oven-dried at 50°C, until the weight stopped fluctuating, for about 72 hours. Next, they were powdered using a grinder (Hanil Ultra-Power Mixer 3.2L-650W, Korea) and then were sieved 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 (vigorous, gentle stirring) for 3 hours at room temperature (approximate at 22°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 Whatman #1 filter paper. The alcohol was removed from the extracted solution using a rotary evaporator (R-100 rotary evaporator, Buchi, Switzerland). Thereafter, the extract was held at -70°C prior to lyophilizing at -55°C (Ilshin freeze dryer, Korea) until completely dry for about 3 days. All samples were then kept in a refrigerator at 2°C before analyzing the phytochemical compounds, antioxidant activity and cytotoxicity.

2.3 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/FIR 400, USA). The FT-IR spectra was 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.

2.4 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) FC reagent in deionized water was added, then vortexed for 30 sec and incubated in a dark at room temperature for 30 min. The absorbance was then measured at 750 nm using a UV spectrophotometer (UV 1601, Shimadzu, Australia) against a blank with the same preparation by only replacing 100 µL of FC 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 gram of dry leaves (mg GAE g^{-1}).

2.5 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 trichloroacetic acid (TCA) 10% (100 mg/mL) and mixed well. 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 FC 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 the same preparation by only replacing 0.2 mL of FC 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 were calculated based on the standard curve.

2.6 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 then 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 a blank with the 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 gram of dried leaf powder (mg QE g^{-1}).

2.7 DPPH Radical Scavenging Assay

DPPH radical-scavenging ability was measured using the method of Huang et al. (2006) [11] with some modification. Different concentrations (31.2, 62.5, 125, 250, 500, 750, 1000 and 2000 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, AA and quercetin (Sigma) were used as the 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.

2.8 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 for 10 min for reaction at RT. The absorbance was then measured at 562 nm, and EDTA (Sigma) was used as a positive reference. 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.

2.9 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°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 with high values regarded as high reducing power, and ascorbic acid was used as a positive control.

2.10 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°C for 90 min. After, the samples were cooled to RT and 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}).

2.11 Cells Culture and Cytotoxicity Assay

The cytotoxicity of leaf 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-well plates at a density of 2×10^4 cells per well and incubated for 24 hours 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 were performed to obtain the final concentration of 31.2, 62.5, 125, 250, 500, and 1000 µg/mL. The dilutions of the extracts were used to treat the MDCK cells and incubated for 48 hours at 37°C, 5% CO_2. Then CCK-8 kit reagent was added and after the incubation time (1 hour, 37°C, and 5% CO_2), and the absorbance was measured at 450 nm using a microplate reader

(Synergy, Bio-Tek, VT, USA). 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 (extract-treated cells), background (medium/extracts without cells), and the control (control medium with cells), respectively. Nonlinear concentration−response curves were plotted, and the half-maximal cytotoxic concentration (CC_{50}) was calculated.

2.12 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 (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).

3. RESULTS AND DISCUSSION

3.1 Effects of Solvents on Phytochemical Components of *Mulberry mistletoe* Leaf

3.1.1 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 findings 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 its antioxidant activity [15]. In addition, a previous study by 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 activity [16]. Thus, these compounds from the extracts of *Mulberry mistletoe* leaves may function as antioxidants and antimicrobial agents.

3.1.2 Total phenolic compound (TPC)

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

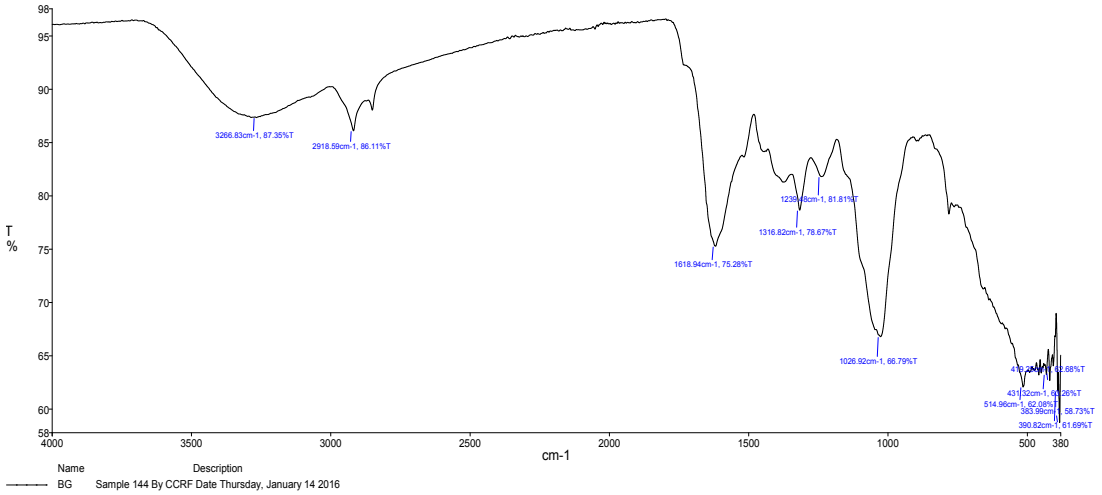

Fig. 1. FT-IR analysis of *Mulberry mistletoe* leaves crude powder. The spectrum was analyzed in the Spotlight 400 FT-IR, Perkin Elmer systems at the wavelength ranging from 4000 to 400 cm^{-1} and the peaks were analyzed using the Perkin Elmer Spectrum Version 10.03.05.

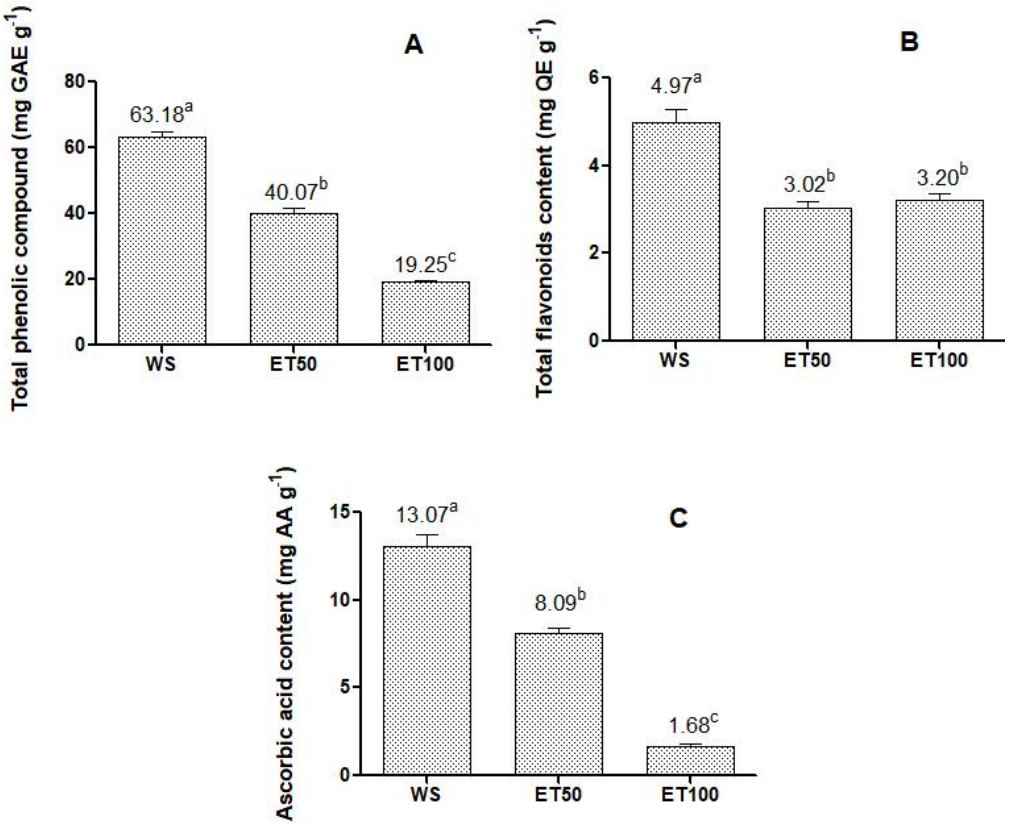

Fig. 2. The phytochemical equivalent of *Mulberry mistletoe* leaves as affected by different solvents. Total phenolic content (A), Total flavonoids content (B), and Ascorbic acid content (C). Values (Mean ± SD of triplicate) with different superscript letters ($^{a-c}$) above bars indicate significant difference from one another at $p < 0.05$ (Duncan's test). GAE: gallic acid equivalent; QE: Quercetin equivalent; AA: Ascorbic acid equivalent

3.1.3 The total flavonoids content (TFC)

It had been stated by earlier observation that the potential antioxidant activity of flavonoids is related to the chemical structures, which contain multiple hydroxyl group substitutions between 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 leaves by different extracts is shown in Fig. 2B, with the values varied from 3.02 to 4.97 mg QE g^{-1}. The highest value was in WS, while no significant difference was shown between ET50 and ET100, and those values were lower than WS. Thus, pure water was more appropriate for the extraction of TFC than those of ethanol from this leaves.

3.1.4 The content of ascorbic acid (AA)

AA from the extracts of *Mulberry mistletoe* leaves is shown in Fig. 2C. 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 a more efficient means for extraction of ascorbic acid content from the leaf than a water-ethanol system. It could be explained that because of ascorbic acid is a water-soluble complex, more AA was contained in the WS extract. Additionally, based on the results, it can be indicated that the ascorbic acid 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.

3.2 *In vitro* Antioxidant Activities

Many previous studies have shown that phytochemicals from plants and vegetables are believed to provide potential antioxidant benefits. Also, it is known that the bioactive compounds such as phenolics and flavonoids produce a broad spectrum of unique biological effects. Still, much is remaining to find out new sources and new methods to assess and isolate antioxidants from natural materials 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 from different plant extracts must be measured using numerous *in vitro* assays for different mechanisms in order 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.

3.2.1 DPPH radical scavenging ability

The DPPH ability of three different extracts from *Mulberry mistletoe* leaves by percent inhibition is presented in Fig. 3A and 3E. The results show that *Mulberry mistletoe* leaves extracts have potent free radical scavenging activity as compared to quercetin or AA. Since DPPH radical scavenging ability is one of the most commonly used methods to evaluate the antioxidant activity of various sources, herein we investigated the DPPH ability of leaf extracts of different concentrations. As shown in Fig. 3E, the DPPH values ranged from 20.96 to 77.67% for WS and 5.42 to 74.75% for ET50, whereas the DPPH values for quercetin were from 59.79 to 91.46% (wih 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 and 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 quercetin showed the lowest value at 27.14 µg/mL. Our results were different from previous findings, which stated that all extracts obtained by using a pure and aqueous organic solvent gave stronger DPPH ability than the water extract [18,22]. Changes in solvent polarity alter its ability to dissolve a selected group of antioxidant compounds and influence the antioxidant activity estimations. Thus, it can be inferred that using solvents with higher in polarity is considerably more efficient for extracting of radical scavenging compounds from this plant.

3.2.2 Ferrous ion-chelating ability (FIC)

The results of the FIC assay were plotted as percentage chelating effect by various solvent extracts are shown in Fig. 3B. The FIC of various extracts from *Mulberry mistletoe* leaves followed the order ET80 ≈ pure water > ET50. In fact, pure water extract of *Mulberry mistletoe* leaves showed strong FIC ability (100.58%), which was similar in value to ET100 (101.56%). Interestingly, the results showed that ET50 had lower chelating activity than pure water and ET100 (p < 0.05). This might be due to the complex composition of *Mulberry mistletoe* leaves, which contained a variety of antioxidant components with differing in polarity and various mechanisms with higher proportions of

hydrophilic compounds. In this case, it can be observed that pure water was more favorable in the extraction of the ion chelating compounds in this leaf as compared to the other solvents. Our results were similar to the study of Yeşiloğlu and Şit (2012), who showed that the percentages for the ion chelating capacity of water extract were higher than those of ethanol or acetone [23]. It suggested that pure water might be a good solvent for extraction of the ion chelating components of *Mulberry mistletoe* leaves.

3.2.3 Reducing power ability (RDP)

The RDP of various extracts is presented in Fig. 3C, which shows that the reducing power ability was dependent on the solvents used (p<0.05). In

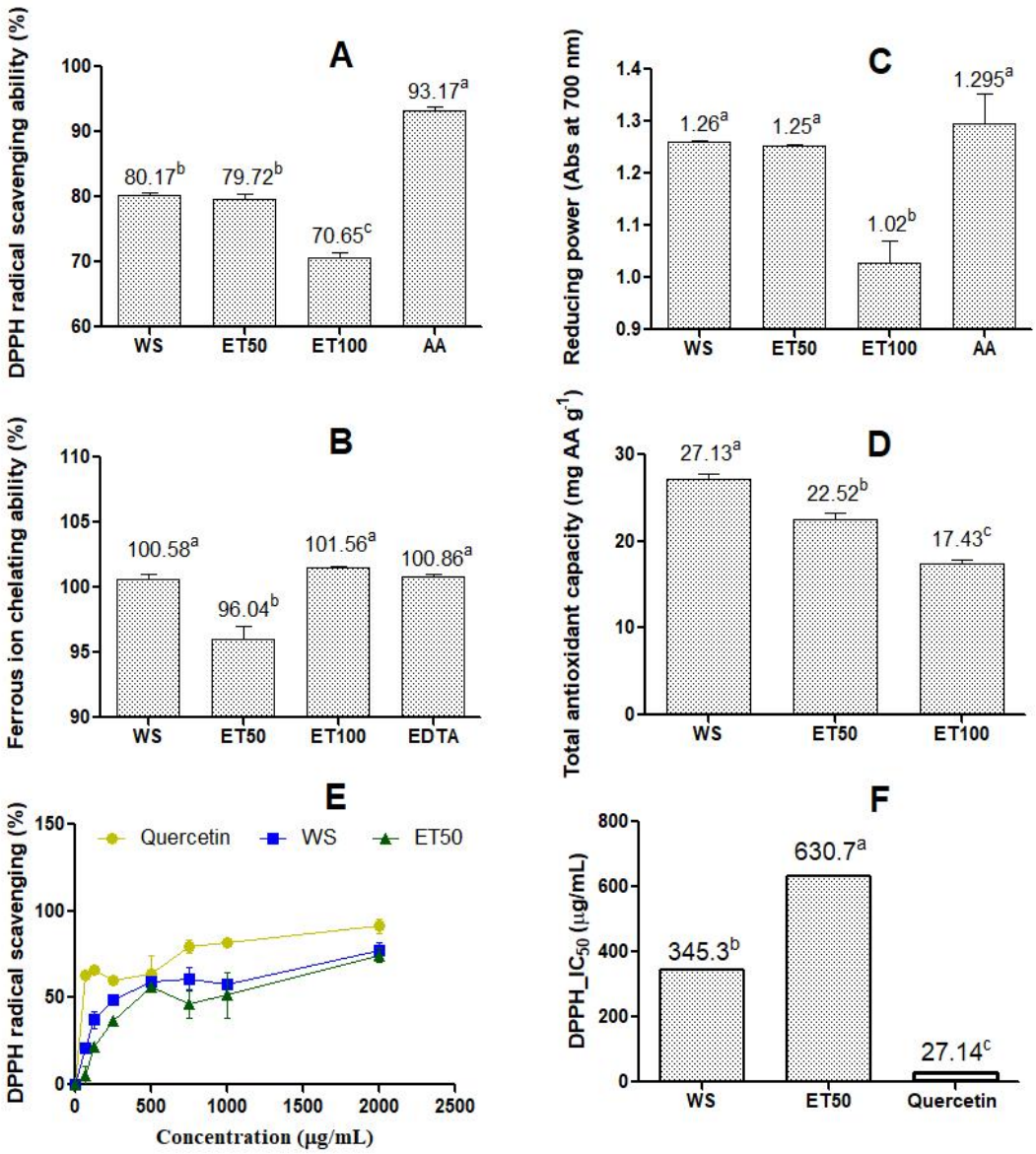

Fig. 3. Antioxidant activities of leave extracts. DPPH radical scavenging ability (A), Ferrous ion-chelating ability (B), Reducing power ability (C), Total antioxidant capacity (D), DPPH radical scavenging ability at different concentration (E) and together with its IC_{50} values (F). Values (Mean ±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

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 findings of Anwar and Przybylski (2012) [24], who reported that RDP was the highest in pure methanol extract, which had higher values than 80% ethanol and 80% methanol extracts. The difference between these results could be explained that may be due to the variety of the plant materials with various mechanisms that might contribute to oxidative processes.

3.2.4 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 results are 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 changes in polarity and the vapor pressures of solvents might significantly influence their antioxidant capacities. Several previous studies have measured the effects 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 strongest total antioxidant capacity. It could be explained that almost all antioxidant compounds in these leaves were mostly water-soluble components (hydrophilic groups). On the other hand, these results confirmed that there is a good correlation between TPC and TAC. Therefore, based on the results, it could be revealed that phenolic compounds of the *Mulberry mistletoe* leaf extracts would have the highest contribution to the total antioxidant capacity.

Table 1. Relationship between antioxidant assays and presence of phytochemical 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	

(r values; n = 3), Pearson correlation (2-tailed)
**. Correlation is significant at the p<0.05 level*

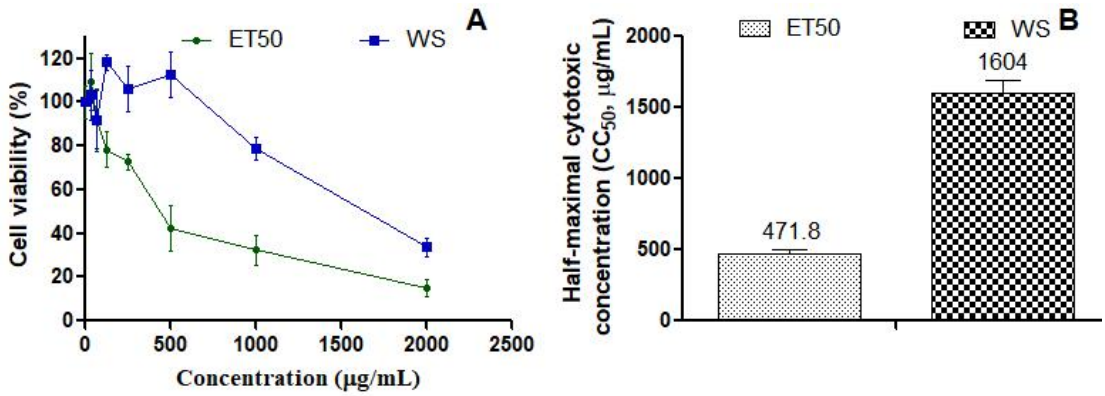

Fig. 4. Cytotoxicity of *Mulberry mistletoe* leaves extracts on MDCK cells. MDCK cells were treated with different concentration of *Mulberry mistletoe* extracts for 48 hours, and CCK-8 kit was added to measure cells viability. Cell viability was expressed as a percentage of the viability of Mock (blank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

3.2.5 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 TPC and TAC are highly correlated (r=0.998, $p<0.05$). This suggests that TPC is the dominant contributor to the antioxidant activity of the leaf extract. This result is in agreement with Kchaou et al. (2013), who reported the good correlation between total phenols analysis and antioxidant assays [3]. The results showed that RDP was linearly positively correlated to DPPH (r = 0.997, p < 0.05). However, in the case of FIC, it was a weak correlation. Moreover, our results showed that FIC ability and phytochemicals (TPC, TFC, and AA) are reversible or have no relationship to each other, as observed with r values -0.136, 0.426, and -0.238, respectively (Table 1). Therefore, it can be inferred 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 range of secondary metabolic compounds with differing in polarities and various mechanisms.

3.3 Cytotoxicity of *Mulberry mistletoe* Extracts

As a result is shown in Fig. 4A-B, *Mulberry mistletoe* extracts changed effectively 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 doses 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* has lower cytotoxicity than ET50 extract at certain concentrations. The difference in the cytotoxic dose of leaf extracts may in part be due to the specific compound of phytochemical characteristics of various solvents, as shown in the results mentioned above.

4. CONCLUSION

The results showed a possible influence of extraction solvents on the phytochemical components and antioxidant activities of *Mulberry mistletoe* leaves. Pure water was shown to retrieve higher total phenolic compounds and flavonoids content, maintained antioxidant activity, and have lower cytotoxicity than pure ethanol. 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, antiobesity, antibacterial, antiviral and anticancer in both *in vitro* and *in vivo* are still required.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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