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Original Article

Phytochemical constituents and antioxidant activity of some medicinal plants collected from the Mekong Delta, Vietnam

Tran Thanh Men[1], Nguyen Dinh Hai Yen[2], La Thi Kim Tu[1], Tran Ngoc Quy[3], Nguyen Thi Kim Hue[1], Do Tan Khang[3*]
[1]College of Natural Sciences, Can Tho University, Can Tho city, 94000, Vietnam
[2]Kyoto Institute of Technology, Kyoto 606-8585, Japan
[3]Biotechnology Research and Development Institute, Can Tho University, Can Tho city, 94000, Vietnam

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**Corresponding author email:*
dtkhang@ctu.edu.vn

Abstract

Kaempferia galanga L., *Morinda citrifolia* L., *Morus acidosa* Griff, and *Momordica charantia* L. are medicinal plants that are widely distributed in the Mekong Delta area of Vietnam. This study was meant to examine phytochemical constituents and evaluate the antioxidant activity of ethanol extracts from these four plants. DPPH, ABTS, RP and TAC were applied to assess their potential *in vitro* antioxidant activity. *Drosophila melanogaster* flies were experimental objects used to evaluate *in vivo* antioxidant activity. Based on the data analyzed, extract of the plant *Momordica charantia* L. was resulting in the greatest phenolic content at 64.63 ± 1.51 mg GAE/g extract and flavonoid content at 196.52 ± 5.76 mg QE/g extract. The analyzed result of antioxidant activity through ABTS, RP, and TAC methods also displayed the highest IC_{50} values of this plant extract, which was 60.02 ± 2.51 µg/mL, 69.64 ± 2.74 µg/mL, and 110.95 ± 1.65 µg/mL, respectively. Meanwhile, a maximum IC_{50} value of 168.30 ± 6.97 µg/mL, which was collected through the scavenging activity by DPPH, was introduced in the extract of *Morus acidosa* Griff. However, this value was insignificantly different as compared with the IC_{50} values of *Morinda citrifolia* L. extract at 172.30 ± 5.23 µg/mL and *Momordica charantia* L. extract at 188.20 ± 7.52 µg/mL. Under paraquat and H_2O_2-induced oxidative stress conditions, the adult fly, which was fed on extracts, had increased parameters of mean lifespan, 50% survival time, and maximum lifespan. These findings showed that *Momordica charantia* L. extract had the highest *in vivo* antioxidant activity among the investigated plant extracts. It could be concluded that *Kaempferia galanga* L., *Morinda citrifolia* L., *Morus acidosa* Griff, and *Momordica charantia* L., especially *Momordica charantia* L., are potential medicinal plants containing many antioxidant compounds.

Keywords: ABTS, Antioxidant, DPPH, *Drosophila melanogaster*, *Momordica charantia* L., RP, TAC

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Introduction

The species of reactive oxygen (ROS) can be defined as those reactive molecules related to the cause of many diseases such as cancer, cardiovascular, cataract, asthma, hepatitis, and immunodeficiency (Lee et al., 2004). Antioxidant compounds, including phenolics and flavonoids, work to eliminate the free radicals, for example, hydroperoxide, peroxide, and lipid peroxide, thereby preventing the formation of oxidative stress causing degenerative diseases (Wu et al., 2011). The compounds extracted from medicinal plants possess various biological substances and therapeutic uses, such as anticancer, anti-inflammatory, anti-diabetic, and anti-aging (Goel et al., 2012). Approximately 80% of developing countries use natural plants to treat different diseases (Rakotoarivelo et al., 2015; Ashraf et al., 2021). Many herbal species contain vitamins, carotenoids, terpenoids, polyphenols, alkaloids, tannins, saponins. These compounds are shown to have biological activities and therapeutic properties in humans (Sharma et al., 2011).

The Mekong Delta region is well-known for its diverse and abundant medicinal plants with antioxidant properties, essential in treating different diseases. In this study, *Kaempferia galanga* L., *Morinda citrifolia* L., *Morus acidosa* Griff, and *Momordica charantia* L., which are selected from the Mekong Delta of Vietnam, are the four medicinal plants used to determine and evaluate the existence of *in vitro* and *in vivo* antioxidant activity. *Drosophila melanogaster* (*D. melanogaster*) fruit flies were used to assess medicinal plants containing antioxidant activity. This species of flies have been used in the laboratory since 1991, thanks to its beneficial characteristics in biological research. Some previous studies indicated that approximately 75% of human disease genes are homologous to the genes of *Drosophila*. Moreover, *Drosophila* has been successfully applied for *in vivo* modeling of higher metazoans aging and antioxidant studies (Bier, 2005, Padalko et al., 2018).

Material and Methods

Plant collection and identification

Kaempferia galanga L. rhizome (*K. galanga*), *Morinda citrifolia* L. leaves (*M. citrifolia*), *Morus acidosa* Griff leaves (*M. acidosa*), and *Momordica charantia* L. leaves (*M. charantia*) were collected from the Mekong Delta area of Vietnam during June 2019. These plant samples were authenticated and stored in the Botanical Laboratory by Dr. Nguyen Thi Kim Hue, Department of Biology, Can Tho University, Vietnam.

Preparation of the extract

After being collected, plant samples were shade dried in the dark. Dried samples were allowed for grinding in an electric blender to get powder before subjecting to solvent extraction. 100 mL of ethanol 96% (India) was added to 10 g of the fine powder to prepare the extract. The mixture was then remained at room temperature for 48 hours. Whatman No.1 (Camlab, UK) was taken to filter the extracts. A concentration process was then performed at 50°C using an evaporation device. The final extracts were collected and stored at 4°C for further analysis (Haruna et al., 2020).

Qualitative analysis of phytochemical constituents

The chemical compounds containing in plant extracts were estimated with the method reported by Haruna et al. (2020) with some modifications.

Alkaloids: A solution of 1.36 g of mecuric chloride ($HgCl_2$) dissolved in 60 mL dH_2O and a solution of 5g of potassium iodide (KI) dissolved in 20 mL dH_2O were combined (Wagner's reagent). Wagner's reagent at a small volume was treated with 1 mL of extract. The red-brown precipitate formation was considered to have alkaloids.

Saponins: A solution prepared by adding 5 mL of distilled water to 1 mL of the extract in a test tube, followed by supplying 3 - 4 drops of ethanol. After stoppering and shaking vigorously for 5 min, the test tube was stayed still for 15 min to check for the honeycomb froth that was considered a determination of saponin presence.

Flavonoids: A blackish-red color formed after adding a few drops of ferric chloride solution to the plant extract indicated that there were flavonoids.

Phenolics: Concentrated sulfuric acid was added to the extract. The mixture was observed for yellow or red-brown color as the positive results for phenolics.

Tannins: Five drops of gelatin 1% were added to 2 mL of extract solution. Precipitation reaction introduced the presence of tannins.

Quantitative analysis of the total amount of phenolic and flavonoid

The reagent of Folin-Ciocalteu (Merk, China) was applied to identify the total amount of phenolic

through the method introduced by Singleton et al. (1999) with some modifications. A reaction mixture included 250 µL ethanol extract, 250 µL of H_2O, and Folin-Ciocalteu reagent were prepared, followed by adding 250 µL of sodium carbonate (Na_2CO_3) 10%. After that, the mixture was taken for incubation at 40°C for 30 min to observe the sample absorbance at 765 nm. The standard used in this method was gallic acid (Merk, Germany). The determination of the total phenolic content was based on the extrapolation of the calibration curve, which was formed by preparing the gallic acid solution. The data were reported by GAE mg/g of the extract that meant the mg gallic acid equivalents per gram of extract.

The method presented by Bag et al. (2015) with some modifications was considered the basis for determining the total flavonoid amount. The reaction mixture, including 200 µL of ethanol extract, 200 µL of distilled water, and 40 µL of sodium nitrite ($NaNO_2$) 5%, was prepared. The mixture was kept in the condition of room temperature for 5 min, followed by adding 40 µL of aluminum chloride ($AlCl_3$) 10% and 400 µL of NaOH 1 M. It continued to be incubated for 6 min to observe the sample absorbance at 510 nm. Quercetin (Merk, China) was taken to be the standard in this method. The total amount of flavonoid was estimated based on the extrapolation of the calibration curve, which was formed by preparing a quercetin solution. The data analyzed were expressed as QE mg/g extract, which meant mg quercetin equivalents per gram of extract.

In vitro antioxidant activity

Scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals

The determination of the antioxidant property of ethanol extracts was based on the DPPH method mentioned by Sharma and Bhat (2009) with few modifications. 100 µL of DPPH ($6x10^{-4}$ M) (Sigma, Germany) combined with 100 µL of ethanol extracts at different concentrations. The solution was stirred thoroughly and kept in the dark at room temperature for 60 min. The mixture absorbance was measured spectrophotometrically at 517 nm. Gallic acid was used to be the standard. The determination of the DPPH radical's reduction ability depended on the decrease of its absorbance at 517 nm. Half maximal inhibitory concentration (IC_{50}) was determined with a standard curve of y = ax + b. The lower value of IC_{50} showed a higher antioxidant capacity (Okoh et al., 2014).

2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) scavenging activity

$ABTS^{\bullet+}$ is known as a stable free radical in blue, absorbing lights at 743 nm. The presence of antioxidant compounds inhibits the formation of $ABTS^{\bullet+,}$ leading to a disappearance of the blue color. This process was described by Nenadis et al. (2004). 7 mM ABTS at the volume of 5 mL was mixed with the volume of 88 µL 140 mM potassium persulfate for preparing the ABTS reagent (Sigma, Germany). The reagent was then incubated in the dark at the condition of room temperature for 16 hours. After processing in the spectrophotometer to reach the optical density of 0.7±0.05 (1:44, v/v) at 734 nm, the neutralization activity of $ABTS^{\bullet+}$ free radical was carried out by adding 990 µL $ABTS^{\bullet+}$ to 10 µL of ethanol extracts. The mixture was kept for 6 min before its measurement at 734 nm.

Reducing power assay

The reducing power (RP) assay was applied based on the description of Oyaizu (1986) and Padma et al. (2013). Different concentrations of ethanol extracts at a volume of 0.5 mL were combined with 0.2 M phosphate buffer (pH 6.6) (0.5 mL) and potassium ferricyanide $K_3Fe(CN)_6$ 1% at 0.5 mL. Incubate the mixture at 50°C for 20 min before adding 0.5 mL of 10% trichloroacetic acid (Sigma, Germany). It was then taken to centrifuge for 10 min at 3000 rpm. A volume of 0.5 mL of the top layer was combined with distilled water (0.5 mL) and of $FeCl_3$ 0.1% (0.1 mL). The mixture absorbance was allowed to be measured at 700 nm.

Total antioxidant capacity (TAC)

The reduction of molybdate (Mo) VI to Mo V using the plant extracts that possess antioxidant constituents is the basic principle to examine the antioxidant capacity through the assay of phosphomolybdenum. The method reported by Prieto et al. (1999) was used to evaluate the antioxidant activity of all extracts. Different concentrations of 100 µL of the extracts were combined with 900 µL of phosphomolybdenum reagent solution prepared by mixing 28 mM sodium phosphate with 0.6 mM H_2SO_4 and 4 mM of ammonium molybdate. The mixture incubation was performed at 95°C in the water bath for 90 min. After heat treatment, the reaction mixture was placed for cooling at room condition and taken for the spectrophotometric analysis at 695 nm.

***In vivo* antioxidant activity**

Fly stock used in this study was Canton-S (CS) wild-type *D. melanogaster* (Bloomington, USA). The male flies, which were newly emerged, were fed on the standard food or diet supplying 0.5 mg/mL of extracts (medicinal plant treatments). 0.05 mg/mL of gallic acid was used as a standard (gallic acid treatment). An amount of ethanol 96% was added to the cultured medium of the control group (control treatment) to check the probable effect of ethanol used in the preparation of plant extracts. Flies were maintained under the laboratory condition at 25 ± 1.2°C, with a relative humidity of 70 - 80% and a photoperiod of 12:12 (light/dark). All experiment groups were moved to the same fresh medium every two days. On day 30, all of the fruit flies in the experiment groups were starved for 2 hours in empty vials which were prepared with distilled water-soaked filter paper. After that, they were taken to separate vials with H_2O_2 10% – saturated filter paper (Sigma, China) or 20 mM paraquat (Sigma, China), which were prepared with a sucrose solution of 6%. The survivals of flies were recorded every 4 hours. The maximum lifespan in this study was calculated as the average lifespan of the 10% longest surviving flies (Peng et al., 2012).

Statistical analysis

The collected data was displayed as the average ± standard deviation from triplicate determinations. These numbers were treated using the statistical method called Analysis of Variance (ANOVA) (Fisher, 1925). Statistical comparison of means followed by the Tukey's test with $p < 0.05$ were considered to be statistically different. Minitab software (Version 16) was applied to carry out all statistical analyses.

Results

Qualitative determination of phytochemical constituents

The phytochemical analysis of the four samples is shown in Table 1. From the qualitative findings presented in Table 1, the ethanol extracts of *M. citrifolia* and *M. charantia* leaves had alkaloids, flavonoids, phenolics, saponins, and tannins. Alkaloids were absent in *M. acidosa* extract; saponins were not present in the extract of *K. galanga*. The study provided evidence for more phytochemical constituents in the ethanol extracts of *M. citrifolia* and *M. charantia* leaves compared with the other two plant species.

Table-1: Qualitative phytochemical analysis

Medicinal plants	Alkaloids	Flavonoids	Saponins	Tanins	Phenolics
M. citrifolia	+	+	+	+	+
K. galanga	+	+	-	+	+
M. charantia	+	+	+	+	+
M. acidosa	-	+	+	+	+

+ present; - absent

Quantitative analysis results of phytochemical constituents

Gallic acid is an organic acid classified as a phenolic compound. The calibration curve of gallic acid determined the total amount of phenolic. The standard curve of quercetin, a compound belonging to the flavonoid groups, was used to determine the flavonoid content. These phytochemical compounds are shown in Table 2.

Table-2: The total amount of phenolic and flavonoid in the four medicinal plant extracts

Medicinal plants	Phytochemical constituents	
	Phenolic (mg GAE/g extract)	Flavonoid (mg QE/g extract)
M. citrifolia	$27.87^{d} \pm 1.80$	$31.30^{c} \pm 3.91$
K. galanga	$55.58^{b} \pm 0.54$	$56.16^{b} \pm 3.15$
M. charantia	$64.63^{a} \pm 1.51$	$196.52^{a} \pm 5.76$
M. acidosa	$46.74^{c} \pm 4.14$	$186.45^{a} \pm 9.83$

The content of phenolics and flavonoids in medicinal plants are in standard equivalents. Values are the average mean ± standard deviation analyzed in three replications. A significant difference is presented in different letters of mean values with 95% level of confidence. GAE: Gallic acid equivalents; QE: Quercetin Equivalents.

Phenolic content: The result of the phenolic contents was presented in mg of GAE per gram of the extract (mg GAE/g). With the value of 64.63 ± 1.51 mg GAE/g extract obtained, *M. charantia* extract showed the highest phenolic content while the *M. citrifolia* extract had the lowest value of 27.87 ± 1.80 mg GAE/g extract. The phenolic content collected from *M. charantia* and *K. galanga* extracts were 64.63 ± 1.51 mg GAE/g extract and 55.58 ± 0.54 mg GAE/g extract.

The data analyzed identified a significant difference between the extracts from the four plants ($p < 0.05$) (Table 2).

Flavonoid content: The flavonoid content of ethanol extracts from the medicinal plants was determined with reference to the standard quercetin and presented as its equivalents (mg QE/g extract). The observation result also showed that the flavonoid contents from *M. charantia* and *M. acidosa* were considered to be significantly higher (196.52 ± 5.76 mg QE/g extract and 186.45 ± 9.83 mg QE/g extract, respectively) than the other two plant extracts ($p < 0.05$). *K. galanga* and *M. citrifolia* extracts had flavonoid contents with the values of 56.16 ± 3.15 mg QE/g and 31.30 ± 3.91 mg QE/g, respectively (Table 2).

In vitro antioxidant activity

The DPPH, ABTS, RP, and TAC assays were applied to evaluate *in vitro* antioxidant activity of plant extracts compared to known antioxidants (gallic acid), and their respective concentrations scavenged 50% of the radicals (IC_{50}) are presented in Table 3.

Table-3: *In vitro* antioxidant effect of gallic acid and plant extracts

Medicinal plants	The IC_{50} value of gallic acid and plant extracts (µg/mL)			
	DPPH	ABTS	RP	TAC
Gallic acid	3.60^{d} ± 0.30	0.44^{e} ± 0.01	0.68^{e} ± 0.03	25.35^{d} ± 0.19
M. citrifolia	172.30^{b} ± 5.23	121.50^{b} ± 4.23	86.06^{b} ± 9.64	116.56^{b} ± 1.38
K. galanga	240.80^{a} ± 5.50	151.64^{a} ± 2.51	116.49^{a} ± 4.76	214.80^{a} ± 6.42
M. charantia	188.20^{b} ± 7.52	60.02^{d} ± 2.51	69.64^{c} ± 2.74	110.95^{b} ± 1.65
M. acidosa	168.30^{b} ± 6.97	99.53^{c} ± 2.44	41.12^{d} ± 3.24	110.11^{b} ± 1.56

Values are mean ± standard deviation of three replications. A significant difference is presented in different letters of mean values with 95% level of confidence.

In the DPPH assay, all plant extracts showed a high concentration of IC_{50} values. The highest one was the extract of *K. galanga* rhizome with 240.80 ± 5.50 µg/mL. The IC_{50} values from the other three plant extracts were a bit lower, which were 172.30 ± 5.23 µg/mL for *M. citrifolia*, 188.20 ± 7.52 µg/mL for *M. Charantia*, and 168.30 ± 6.97 µg/mL for *M. acidosa*. The ABTS, RP, and TAC assays presented the IC_{50} values much lower than those in the DPPH assay. In the ABTS method, the lowest IC_{50} value from *M. charantia* extract was 60.02 ± 2.51 µg/mL; in the RP method, *M. acidosa* extract presented its value of 41.12 ± 3.24 µg/mL. In the total antioxidant capacity (TAC) method, the extract of *K. galanga* showed the highest IC_{50} value of 214.80a ± 6.42 µg/mL, the other lower IC_{50} values from *M. citrifolia*, *M. charantia*, and *M. acidosa* extracts showed the indifference at 116.56 ± 1.38 µg/mL, 110.95 ± 1.65 µg/mL, and 110.11 ± 1.56 µg/mL, respectively ($p > 0.05$).

Table-4: *In vivo* antioxidant effect of gallic acid and plant extracts under 20 mM paraquat condition

Medicinal plants	Life expectancy (hour)	50% survival (hour)	Maximum lifespan (hour)
Control	14.33^{f} ± 1.42	12.0^{c} ± 2.0	20.67^{d} ± 2.01
Gallic acid	30.27^{a} ± 0.64	24.67^{a} ± 1.16	59.0^{a} ± 1.0
M. citrifolia	15.93^{ef} ± 0.9	15.33^{bc} ± 0.58	22.33^{d} ± 2.52
K. galanga	18.4^{de} ± 0.69	15.67^{c} ± 0.58	29.0^{c} ± 1.0
M. charantia	22.4^{bc} ± 0.35	16.33^{b} ± 1.53	42.33^{b} ± 3.06
M. acidosa	20.2^{cd} ± 1.4	14.67^{bc} ± 0.58	32.67^{c} ± 3.01

The treatments were performed in triplicates (three vials, 30 flies per vial). Values are mean ± standard deviation of three replications. A significant difference is presented in different letters of mean values with 95% level of confidence

In vivo antioxidant activity

Paraquat treatment: Chronic paraquat treatment showed that different extracts of medicinal plants caused different effects on the longevity of *D. melanogaster*. Based on the data collected in Table 4, the medium supplementing with 0.05 mg/mL of gallic acid improved the longevity of fruit flies through the parameters of mean lifespan, 50% survival time, and maximum lifespan, compared with the treatment of the control. The flies fed on plant extracts showed the *in vivo* antioxidant activity by increasing the mean lifespan, 50% survival time, and maximum lifespan. The Canton Special (CS) wild-type flies fed on 0.5 mg /mL of M. *charantia* extract expanded the average value of mean lifespan, 50% survival time, and maximum lifespan at 8.07 h 4.33 h, 21.66 h, respectively. The mean lifespan was higher than the control group and other extracts ($p < 0.05$). In contrast, the mean lifespan, 50% survival time, and maximum lifespan of flies fed on *M. citrifolia* extract were higher than the control, but no significant difference was observed.

H_2O_2 treatment: The treatment of hydrogen peroxide also demonstrated similar effects. As the analyzed result in Table 5, flies from the control treatment had an average lifespan of 21.0 ±1.0 h. In contrast, the flies fed on plant extract showed a higher average lifespan from 12 to 18.6 h, presenting *in vivo* antioxidant activity. A diet supplemented with 0.5 mg/mL of *M. charantia* extract led to the longest average lifespan at 18.6 h, the 50% survival at 22.34 h were compared with the result collected from the control group ($p < 0.05$). The 10% survival time of flies fed on *K. galanga* extract showed the longest 24.34 h as compared with the value of the control treatment.

Table-5: *In vivo* antioxidant effects of gallic acid and plant extracts under 10% H_2O_2 condition

Medicinal plants	Mean lifespan (hour)	50% survival (hour)	Maximum lifespan (hour)
Control	$21.0^{e} \pm 1.0$	$15.33^{e} \pm 0.58$	$35.33^{d} \pm 0.58$
Gallic acid	$47.0^{a} \pm 1.0$	$53.0^{a} \pm 1.0$	$62.33^{a} \pm 0.58$
M. citrifolia	$33^{d} \pm 2.91$	$30.33^{d} \pm 2.52$	$42.67^{c} \pm 2.52$
K. galanga	$36.87^{cd} \pm 1.35$	$30.0^{d} \pm 2.0$	$59.67^{a} \pm 0.58$
M. charantia	$39.6^{bc} \pm 1.44$	$37.67^{bc} \pm 0.58$	$54.0^{b} \pm 2.0$
M. acidosa	$37.33^{cd} \pm 2.91$	$36.33^{b} \pm 2.08$	$44.0^{c} \pm 1.0$

The treatment was performed in triplicates (three vials, 30 flies per vial). Values are mean ± standard deviation of three replications. A significant difference is presented in different letters of mean values with 95% level of confidence.

Discussion

Medical plants contain an abundance of bioactive compounds and sources of antioxidant phytochemicals. Trends in medical applications are extracting natural antioxidants from herbals and determining its chemical components for isolation (Xu et al., 2017). The result in Table 1 shows the existence of flavonoids, tannins, and phenolics in the extracts of the four medicinal plants. Specifically, *M. citrifolia*, *M. charantia*, and *K. galanga* had alkaloids; saponin was present in the extracts of *M. citrifolia*, *M. charantia* and *M. acidosa* but was absent in *K. galanga*. Previous studies indicated that plants produced secondary compounds belonging to phenolic, alkaloids, flavonoids, and tannins (Tungmunnithum et al., 2018; Cosme et al., 2020). To identify the secondary metabolite existence, the screening for phytochemicals was subjected to carry on the aqueous extracts of M. citrifolia leaves. The result indicated that *M. citrifolia* leaves had alkaloids, coumarins, flavonoids, tannins, saponins, steroids, and triterpenoids (Serafini et al., 2011). Phytochemical screening result of *K. galanga* rhizome extracts identified the existence of flavonoids, steroids, and terpenoids in the hexane extract; tannins, steroids, and terpenoids in the ethyl acetate extract; all phytochemical constituents except steroids in methanol extract (Sani et al., 2019). Study results on phytochemical constituents concluded that alkaloids, flavonoids, sterols, anthraquinones, and phenols were presented as the main active components in the extracts of *M. charantia* leaves (Annapoorani and Manimegalai, 2013). Wulandari et al. (2019) proved that mature leaves of *M. acidosa* contained phytochemical groups including alkaloids, phenolic, flavonoids, tannin, and terpenes. These mentioned medicinal plants possessed numerous natural compounds with positive biological activities that could be proved in several former studies (Wulandari et al., 2019; Cosme et al., 2020).

Phenolic and flavonoid compounds had antioxidant activities, thus benefiting human health, curing and preventing many diseases (Tungmunnithum et al., 2018). Flavonoid or bioflavonoid is a ubiquitous group of polyphenol substances present in most plants (Miller, 1996). The conclusions of numerous studies have clarified that flavonoids express the strong effects of antioxidant activities (Amic et al., 2007). Shahidi et al. (1992) affirmed the pharmacological properties of flavonoids to their potent antioxidant activity. This study showed that four ethanol extracts contained biological compounds with potential application in antioxidant research. The extract of *M. charantia* leaves was considered outstanding with the highest phenolic content of 64.63 ± 1.51mg GAE/g extract and flavonoid compound of 196.52 ± 5.76 mg QE/g extract. Meanwhile, the phenolic content of 27.87 ± 1.80 mg GAE/g and flavonoid content of 31.30 ± 3.91 mg QE/g from *M. citrifolia* leaf extract were lower compared with the other extracts. Shodehinde et al. (2016) indicated that the total amount of phenolic and flavonoid in methanol extract of *M. charantia* leaves were 52.43 mg GAE/100g extract and 38.75 mg QE/100g extract. That was higher than the aqueous extracts at 46.95 mg GAE/100g extract and 29.46 mg QE/100g extract. Our result illustrated that the ethanol extract from leaves of *M. charantia* contained higher phenolic and flavonoid contents compared with the previous study (Xu et al.,

2017; Cosme et al., 2020).

IC_{50} values determined oxidation resistance; the lower values indicated the better oxidation resistance of medicinal plant extracts. In this study, all ethanol extracts from medicinal plants had antioxidant activities through DPPH, ABTS, RP, and TAC assays, of which *M. charantia* was the extract with the best antioxidant capacity. The lowest antioxidant capacity was from the extract of *K. galanga*. This finding was consistent with the analysis result of phenolic and flavonoid. Ethanol extract from *M. charantia* showed the highest phenolic and flavonoid contents, 64.63 ± 1.51 mg GAE/g extract, 196.52 ± 5.76 mg QE/g extract, respectively. As introduced in multiple reports, phenolic compounds exhibited the inhibition of free radicals, the decomposition of peroxide, metal inactivation, scavenging oxygen in a biological system, and prevented oxidative-related disease (Babbar et al., 2015). In addition, phenolics are known as good compounds donating electrons thanks to their hydroxyl groups' direct contribution to antioxidant action (Bendary et al., 2013). Moreover, Bortolotti et al. (2019) proved that *M. charantia* had many phytochemical constituents, including flavonoid and phenolic compounds. Several studies *in vitro* have evaluated the potential antioxidant activity of *M. charantia* extracts. Pretreatment of neuroblastoma cells with *M. charantia* extracts was found to cut down the H_2O_2-induced cytotoxic oxidative stress by making an increasing ability and reducing H_2O_2-induced activating of signaling pathway regarding NKs, p38, and extracellular signal-regulated kinase (ERK1/2) mitogen-activated protein kinase signaling (MAPK) (Kim et al., 2018).

The data collected in Tables 4 and 5 shows that the lifespan of *Drosophila* was extended in the medium with 0.05 mg/mL of gallic acid in both paraquat and H_2O_2 conditions. Under paraquat condition, the mean lifespan of gallic acid treatment was 30.27 ± 0.64 h, 2.11 times higher than the control; 50% survival time was 24.67 ± 1.16 h, 2.05 times higher than the control, and maximum 10% survival time was 59 ± 1.0 h, 2.95 times higher than the control; whereas, under H_2O_2 condition, these higher values were 2.24 times, 3.46 times and 1.76 times, respectively. This result is consistent with previous studies on the ability to resist oxidative stress of gallic acid (Badhani et al., 2015). Flies fed on diets containing the extracts were observed *in vivo* antioxidant under stress conditions of paraquat and H_2O_2. Among the four plant extracts, *M. charantia* had better *in vivo* antioxidants in both paraquat and H_2O_2 treatments (Table 4 and Table 5). The result of paraquat treatment was presented under three indicators, including average lifespan, time of 50% survival, and maximum 10% survival time. The lifespan of flies fed on *M. charantia* extract was higher than the standard diet by 8.07h, 4.33h, and 21.66h, respectively (Table 4). Moreover, under H_2O_2 conditions, flies fed on food supplementing with 0.5 mg/mL of *M. charantia* extract had a higher mean lifespan, time of 50% survival, maximum lifespan than those using standard food (Table 5). He et al. (2018) proved that *M. charantia* ethanol extract from leaves was valuable for preventing hyperlipidemia and oxidative stress induced by a high-fat diet in a rat model. The average lifespan, time of 50% survival, and maximum lifespan of flies fed on four extracts were lower than gallic acid and had statistical significance. This result is consistent because gallic acid is a standard commercial antioxidant with high purity. In addition, medicinal plant extracts are highly synthetic extracts containing many different compounds that can act as mutual inhibitors. The study results may conclude that the plant extracts have the highest *in vivo* antioxidant activity in both paraquat and H_2O_2 assays.

Conclusion

In conclusion, *M. charantia* could be a good resource of natural compounds with significant antioxidant activity (*in vitro* and *in vivo*). This report was resulted by its high percentage of phenolic and flavonoid contents. Based on the results obtained, further study on the isolation and identification of compounds with biological activity from *M. charantia* is essential.

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Contribution of Authors

Men TT & Khang DT: Conceived idea, designed research methodology, data collection & analysis, literature review, writing and final approval of manuscript

Yen NDH: Designed antioxidant methodology and analyzed the data

Kim Tu LT & Quy TN: Participated actively in the extraction, editing and final approval of manuscripts

Hue NTK: Supervised compilation of data, literature review, data processing, final reading, and acceptance of manuscripts.