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Pharmacognostical standardization, phytochemical analysis, and antioxidant activity of *Musa balbisiana* Colla fruits

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ABSTRACT

Musa balbisiana (MB) fruits are widespread as an herbal medicine worldwide. This study aimed to screen phytochemicals, standardize raw materials and crude extract, and evaluate antioxidant property of MB fruit extracts. Standardization was performed using purity content and chemical characterizations, total polyphenol (TPC) and flavonoid (TFC) contents to assess the quality. The phytochemicals were determined by chemical reactions. DPPH quenching, ABTS cation decolorization, and reducing power assays were selected to investigate antioxidant ability. Results indicated that physicochemical parameters for MB fruits were within prescribed Vietnamese Pharmacopoeia 5^{th} limits. Phytochemical analysis of MB fruits disclosed the presence of volatile oils, lipids, flavonoids, proanthocyanidins, saponins, and triterpenoid . The TPC was 3.92 $\pm$ 0.27 mg GAE/g d. w. (dry weight) and 162.64 $\pm$ 3.39 mg GAE/g d. w. for the raw materials and crude extract respectively while the TFC was 0.138 $\pm$ 0.002 mg QE/g d. w. and 1.222 $\pm$ 0.007 mg QE/g d. w.. Furthermore, crude extract and some of its fractions have a high radical scavenging activity. These initial studies play a pivotal role in the procurement of raw materials and evaluation of the quality of the crude extract, and show antioxidant potential of MB fruits.

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INTRODUCTION

Musa balbisiana Colla belonging to the family Musaceae is widespread as a herbal medicine with great therapeutic potential worldwide. The parts of *M. balbisiana* (such as fruits, leaves, stem, roots, inflorescence) have been proven to have many effects such as diuretic effect and cure edema, antidiabetic, anti-lipidemic properties, detoxify, stimulate digestion, treat the disease of the stomach (Deka, 2018; Deka and Talukdar, 2007), antibacterial activity (Hoe *et al.*, 2015), cure rheumatism and headache (Alam, 1992; Gogoi and Konwar, 2013). Traditional healers have ubiquitously used *fruits* for centuries to treat various diseases such as stomach pain, urinary stones, and osteoarthritis pain. Especially, the ripe fruits are nutritious, used as baby food and are good sources of vitamin C, potassium, riboflavin, and pyridoxine (Barthakur and Arnold, 1990). With the benefits of *M. balbisiana* fruits, the need to create health care

products for people is essential. One of the criteria for preparing phytopharmaceutical preparations should relate to the screening of phytochemicals and standardization of medicinal materials. Phytochemical screening can give an overview of secondary metabolic compounds of the *M. balbisiana* extract in a class of compounds to their activity and offer enormous opportunities for nutraceutical, pharmacological and food additive applications. Standardization of the process of evolving, implementing technical standards, and optimizing drug compatibility, safety, reproducibility, and quality is one of the most crucial criteria in many practical problems, particularly in medicinal products from herbs. Scientific evidence concerning herbal medicinal product standardization could be of great importance to confirm the efficacy, health, and toxicity of herbal medicine (Setyawati *et al.*, 2019).

Furthermore, free radicals are formed in our bodies on a continuous basis as a result of biological oxidation or metabolism and can be related to many diseases, for instance, cancer, diabetes mellitus, osteoarthritis, neurodegenerative diseases, atherosclerosis, and aging (Halliwell and Gutteridge, 1989). In spite of in-built antioxidant mechanisms, the use of natural antioxidants available in diets (particularly in fruits and vegetables) seems more meaningful (Renaud *et al.*, 1998; Temple, 2000). Not only are antioxidants of natural origin helping to promote safety and various effects, but they are also healthier and more environmentally friendly than synthesized antioxidants. Among natural sources of antioxidants, plants are a potential rich source. Natural plant antioxidants may reduce cellular oxidative damage, prevent ageing and other degenerative diseases. Among phytochemicals, two main classes are related to the protective effects of natural antioxidants: polyphenols and flavonoids. They are known as a powerful secondary antioxidant source. Because their side effects are minimal, it seems more interesting to utilize natural preparations for prophylactic and therapeutic medicine (Patel *et al.*, 2010).

Although *M. balbisiana* fruits have many traditional, pharmacological, and nutritive values, to date, the data available on the standardization of plant materials and extract to quality control in the investigation of antioxidant activity for *M. balbisiana* fruit profile is limited. Thus, this study was established to perform standardization of raw materials and extract of *M. balbisiana* fruits to contribute to quality control of medicinal materials and investigate the antioxidant abilities of *M. balbisiana* fruit extracts. The initial survey results are the scientific basis for further research on chemical composition, biological effects as well as the use of this medicinal herb in treatment.

MATERIALS AND METHODS

Medicinal plant preparation

M. balbisiana fruits were collected in May 2019 from Tan Thanh District, Long An Province. The plant samples were identified and verified by MSc. Le Duc Thanh at the Research Center of Ginseng and Medicinal Materials and a voucher specimen (TNDL-QCH-2019) was deposited for *M. balbisiana* Colla. The dried materials were ground into powder and kept in a sealed bag (Sample code: QCH-TTS-2019) at the Research Center of Ginseng and Medicinal Materials in Ho Chi Minh City.

Chemicals and reagents

Folin-Ciocalteu 's reagent, quercetin, gallic acid, DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) and ascorbic acid were purchased from Sigma Co. Ltd, USA, and TLC silica gel 60 F_{254} (Merck). Ethanol was purchased from OPC Pharmaceutical Company. Several other chemicals and solvents of the analytical type were also used in this study.

Preparation of extract and fractions

The material powder was individually extracted with 45% ethanol at a ratio of (1: 20 (g/mL), material: solvent) by the hot extraction method for 60 minutes. This extract was then collected via filtering and then concentrated under reduced pressure by a device called a rotary evaporator at 70 °C to obtain crude extract (CE extract). Liquid-liquid extraction with solvents of increasing polarity was used to achieve fractionated extracts from the crude extract. The fractionated extracts included *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and aqueous fractions, which were denoted F1, F2, F3, F4, and F5, respectively. The crude and different fractions were stored in a refrigerator at a temperature of around 2-8 °C and they were dissolved in a suitable solvent to carry out tests. The extraction yield of crude extract was estimated using the expression,

$$H(\%) = \frac{m \times (1-a)}{M \times (1-A)} \times 100$$

In which H is extraction yield (%), m is mass of the crude extract (g), M is mass of the dried powder (g), a is moisture of the crude extract (%), and A is moisture of the dried powder (%).

The extraction yield of fractionated extracts was estimated using the expression,

$$H(\%) = \frac{m}{M \times (1-A)} \times 100$$

In which H is extraction yield (%), m is mass of the fractionated extract (g), M is mass of the crude extract (g), and A is moisture of the crude extract (%).

Microscopic examination of *M. balbisiana* powder

A small quantity of *M. balbisiana* powder was put on a microscopic slide, then phloroglucinol (0.1% *mass/volume*, 1-2 drops) and concentrated HCl (one drop) were added in turn and it has continued to be covered with a coverslip. The preparation of the slide was suspended in glycerol and investigated under a microscope. The ingredients in medicinal powder and their characteristic structures were observed and their photographs were got using a camera (Ushir and Morankar, 2014).

Preliminary phytochemical screening of *M. balbisiana* fruits

Preliminary phytochemical screening was proceeded to identify the presence of phytochemicals in *M. balbisiana* fruits. The screening was carried out in accordance with Cuilei's method (Ciulei, 1982) with volatile oils, lipids, carotenoids, alkaloids, flavonoids, anthraquinones, anthocyanosides, proanthocyanidins, tannins, coumarins, triterpenoids, saponin, reducing agents, and organic acids.

Physical and chemical parameters determination

Physicochemical parameters (including the loss on drying, the total ash content, and the hydrochloric acid-insoluble ash) of *M. balbisiana* fruits (raw material and the crude extract) were implemented in accordance with the Vietnamese Pharmacopoeia 5^{th} Edition.

Quantitative determination of flavonoids using chemical reactions

The ethanol extract of *M. balbisiana* fruits were tested for the presence of flavonoids by the following chemical reactions,

Alkaline reagent test

Test solution reacts with 10% *w/v* NaOH solution, the reaction solution increases yellow color compared to the original solution.

Ferric chloride test

Test solution reacts with 5% *w/v* $FeCl_3$ solution, the reaction solution is a nut-brown color.

Lead acetate solution test

Test solution reacts with 10% *w/v* $Pb(CH_3COO)_2$ solution, the reaction solution shows a white precipitate.

Shinoda's test

Test solution reacts with magnesium ribbon and concentrated HCl, the reaction solution is the reddish pink color after few minutes.

Thin Layer Chromatography (TLC) Analysis for flavonoids

The stock solution of *M. balbisiana* fruit powder was prepared. The solvent system of Hexane: Ethyl acetate (7: 3, *v/v*)was created and saturated for 30 min before deploying chromatography. Ascending TLC analysis was accomplished using Merck aluminium-backed plates, silica gel 60 F_{254} as a stationary phase, and the aforementioned solvent system was used as a mobile phase to analyze of the extract. Small amount of test solution was detected at about 1.5 cm from the bottom of the TLC plate and then developed for 15 min in the chamber. Then the plate was removed from the chamber and air-dried. Visible bands were surveyed under UV light at 365 nm after a mixture including 2% Aluminium (III) chloride reagent and 5% HCl in ethanol was sprayed on the TLC plate. The colored bands that emerged were marked and the retention factor (R_f) was determined by the formula,

$$R_f = \frac{Dis\tan ce\ travelled\ by\ a\ compound}{Dis\tan ce\ travelled\ by\ the\ solvent\ front}$$

Quantification of total polyphenols content

The total polyphenols content of *M. balbisiana* fruits was estimated by a previously described method using Folin-Ciocalteu's reagent and gallic acid was used as a standard (Alhakmani *et al.*, 2013). The total polyphenols content was done with some modifications. Briefly, 100 μL test extract was mixed with 0.5 mL of Folin-Ciocalteu's reagent in 6.1 mL of distilled water. Then 1.5 mL of 20% *w/v* Na_2CO_3 solution was poured into this mixture right after 5 min, the volume reached up to 10 mL with distilled water. The reaction was kept for 2 hr at room temperature in the dark. The absorbance was measured at 758 nm and all determinations were made in triplicate. Total polyphenol content was calculated using the linear regression equation of gallic acid and expressed as mg gallic acid equivalent per 1 gram of dry weight (mg GAE/ g d. w.).

Estimation of total flavonoids content

The flavonoids content was determined on the basis of the aluminium chloride colorimetric method (Nguyen *et al.*, 2015). Stock standard substance solution was prepared by dissolving 1.0 mg standard substance in 10 mL methanol, then by serial dilutions, including using methanol, the standard quercetin solutions were made. To briefly

illustrate, 1 mL of 2% $AlCl_3$ was added to 0.5 mL diluted extract or standard quercetin solutions separately, and with methanol, the mixture was made up to 10 mL in quantity. Subsequently, the solution was mixed and incubated for 15 min at room temperature. The absorbance of the reaction mixtures was calibrated at λ_{max} value of standard substance with a UV-Vis spectrometer. The measurements were carried out in triplicate. The quercetin with the regression equation showing the relationship between quercetin content and optical density of quercetin standard. Total flavonoids content was estimated using the linear regression equation of the calibration curve and presented as mg quercetin equivalent/g of dry weight (mg QE/g d. w.).

Evaluation of antioxidant activity

DPPH radical scavenging assay

DPPH free radical quenching assay was applied to evaluate the antioxidant activity of *M. balbisiana* fruit extracts based on a previously described method (Alhakmani *et al.*, 2013). Briefly, 4 mL reaction mixture consisting of 0.5 mL of different concentrations of test extract or ascorbic acid (positive control) and 0.5 mL of 0.6 mM DPPH reagent in methanol was incubated at room temperature for 30 min in the dark. After that, the absorbance was acquired at 516 nm and all tests were done in triplicate. The results were expressed as an IC_{50} value for each sample from the proportion of the free radical scavenging activity, which was determined by the expression,

$$\%\ scavenging\ effect\ =\ \frac{Ac - At}{Ac} \times 100$$

In which A_c and A_t are the absorbance of the blank (without test extract) and the sample (with test extract), respectively.

ABTS radical scavenging assay

The ABTS antioxidant test was carried out according to the following description: ABTS solution was prepared by adding a 7 mM ABTS solution to a 2.4 mM potassium persulfate solution of equal volume and then incubating the solution. Fluid in the dark for 16 hours at room temperature. The solution is then diluted by mixing 1 mL of ABTS solution with 50 mL of methanol to obtain an absorbance of 0.706 $\pm$ 0.01 units at 734 nm using a spectrophotometer. Use this solution for testing. 40 μL of the test sample at various concentrations or ascorbic acid (positive control) was mixed with 1160 μL of ABTS solution (1: 29), measure the absorbance at 734 nm after 6 minutes at room temperature. All tests were done in triplicate and an average of each sample was calculated (Nguyen *et al.*, 2017). The results were expressed as an IC_{50} value for each sample from the proportion of the radical quenching activity, which was calculated by the formula,

$$\%\ scavenging\ effect\ =\ \frac{Ac - At}{Ac} \times 100$$

In which A_c and A_t are the absorbance of the blank (without test extract) and the sample (with test extract), respectively.

Antioxidant activity was also assessed through IC_{50} (Half inhibitory concentration), which is the concentration of antioxidant that need to inhibit 50% of free radicals. The IC_{50} value is determined based on the equation illustrating the correlation between the test substance concentration and the percentage of antioxidant activity using Graphpad Prism software.

Reducing antioxidant power (RP) assay

The sample will reduce Fe^{3+} ions in $K_3[Fe(CN)_6]$ to Fe^{2+} ions in $K_4[Fe(CN)_6]$. When $FeCl_3$ is added, Fe^{3+} will react with ferrocyanide ions to form a blue ferrocyanide complex ($K_4[Fe(CN)_6]_3$) (Oyaizu, 1986). Briefly, 0.2 mL of the sample at the concentrations examined was added with 0.5 mL of PBS phosphate buffer solution (pH = 6.6) and 0.5 mL of K_3 $[Fe(CN)_6]$ (carried out in the dark), incubated at 50 ^{o}C, 30 minutes. After that, each tube was cooled and then added with 0.5 mL of 10% trichloacetic acid solution TCA and centrifuge 3000 cycles for 10 minutes. Then, 0.5 mL of the above solution (the supernatant) was taken into a new eppendorf, 0.1 mL of $FeCl_3$ solution and 0.5 mL of distilled water were added. Measuring absorbance at a wavelength of 700 nm, the optical density value OD reflects the reducing ability of the sample. The antioxidant ability of the test sample was judge via optical density and EC_{50} values. The lower the optical density value, the weaker the reduction activity of the sample. EC_5 value is the concentration of effective antioxidant for optical density reaches 0.5, which was calculated through the equation illustrating the correlation between the concentration of the sample and its optical density using Graphpad Prism software.

Statistical analysis of data

The data were statistically analyzed by t-test and expressed in terms of mean $\pm$ SEM (Standard error of the mean) using Graphpad Prism software (version 8, Inc., La Jolla, CA, USA).

RESULTS AND DISCUSSION

Characteristics of *M. balbisiana* fruit powder

The *M. balbisiana* fruit powder was brown in color and smells pretty strong, on microscopical exami-

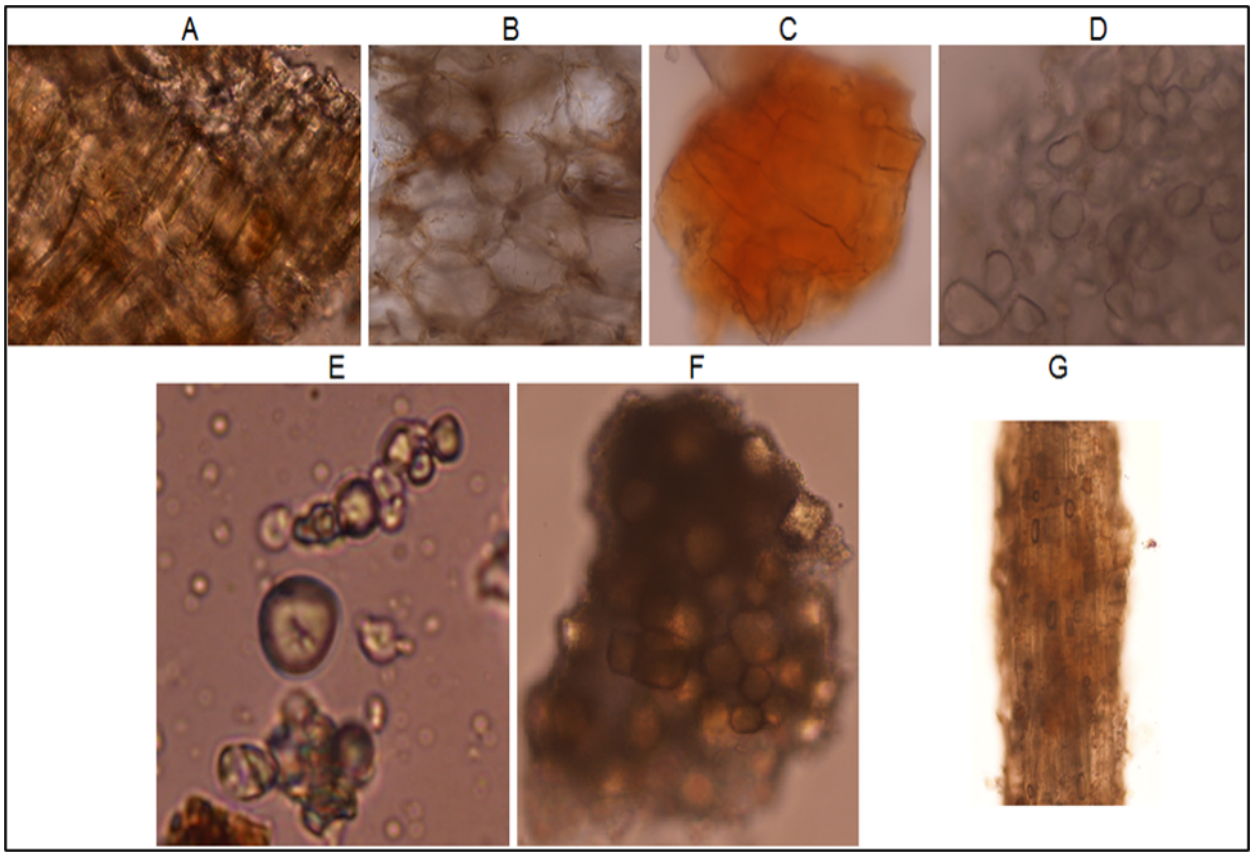

Figure 1: Powder characteristic of *M. balbisiana* fruits

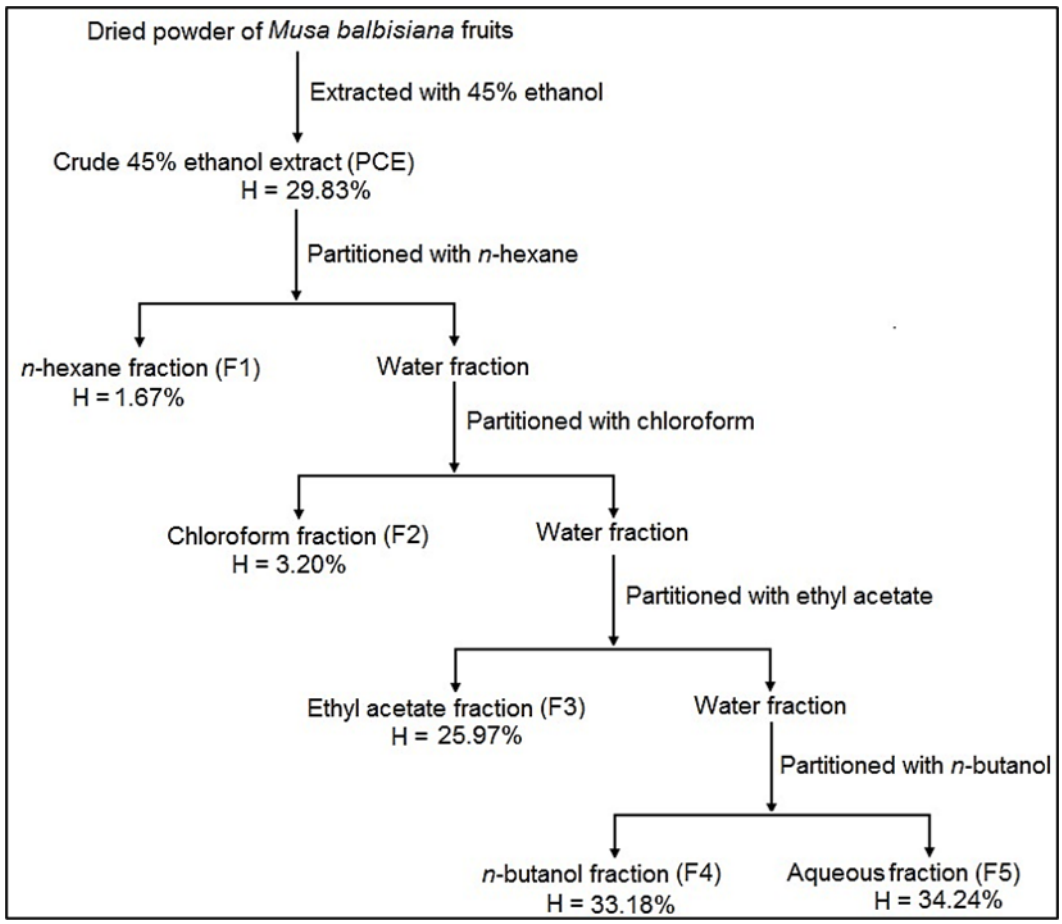

Figure 2: The scheme of extraction and fractionation of *M.balbisiana* fruits

Table 1: The phytochemical analysis of *M. balbisiana* fruits

Metabolites	Diethyl ether	Ethanol	Aqueous	Presence
Lipids	+	-	-	+
Caroteroids	-	-	-	-
Volatile oils	+	-	-	+
Triterpenoids	+	+	+	+
Alkaloids	-	-	-	-
Coumarins	-	-	-	-
Anthraquinone	-	-	-	-
Flavonoids	+	+	+	+
Anthocyanosides	-	-	-	-
Proanthocyanidin	-	+	-	+
Tannins	-	-	-	-
Saponins	+	+	+	+
Organic acid	-	-	-	-
Reductant	-	-	-	-

(-)absent, (+) present

Table 2: Some physicochemical parameters of *M. balbisiana* fruits

Parameters	Raw materials	Crude extract
The loss on drying (%)	11.44 ± 0.24	18.78 ± 0.20
The total ash content (%)	6.72 ± 0.11	12.78 ± 0.06
The hydrochloric acid-insoluble ash (%)	0.60 ± 0.07	ND

ND: Not determined. Mean $\pm$ SEM (n=3)

Table 3: The calibration curve and R^2 value of gallic acid and quercetin standards

Standard	Regression equation	R^2
Gallic acid	y= 0.0097x + 0.0278	0.997
Quercetin	y = 0.0219x – 0.0554	0.998

Table 4: Total polyphenols content and total flavonoids content of *M. balbisiana* fruits

Sample	Total polyphenols content (mg GAE/g d.w.)	Total flavonoids content (mg QE/g d.w.)
Raw materials	3.92 ± 0.27	0.138 ± 0.002
Crude extract	162.64 ± 3.39****	1.222 ± 0.007****

Mean $\pm$ SEM (n=3); ****p < 0.0001 at the same criteria; mgGAE/g d. w., mg gallic acid equivalent/g dry weight, mg QE/g d. w., mg quercetin equivalent/g dry weight

nation, the powder showed the fruit shell fragment was brown consists of rectangular cells arranged in rows, the soft tissue of the flesh of the fruit has a polygonal shape, the seed shell fragment was orange-yellow consists of rectangular cells. Many starchy grains are bell-shaped, oval-shaped, or polygon-shaped, which are individual or clustered include pieces of starch grains size 20-50 μm (40 $\times$). The navel of the starch particle is slightly off-center, the vascular bundles are scattered. Brown latex was formed in blocks or separately (Figure 1). From Figure 1 A – Shell fragment of fruits, B – Soft tissue fragments, C – Shell fragment of seeds, D – Starch granules clustered, E – Starch navels, F – Latex, G – Vascular bundles.

Preliminary phytochemical screening of *M. balbisiana* fruits

Phytochemical screening investigated gives an idea concerning the chemical nature of the constituents that have biological effects present in extracts of medicinal materials. Preliminary screening of phy-

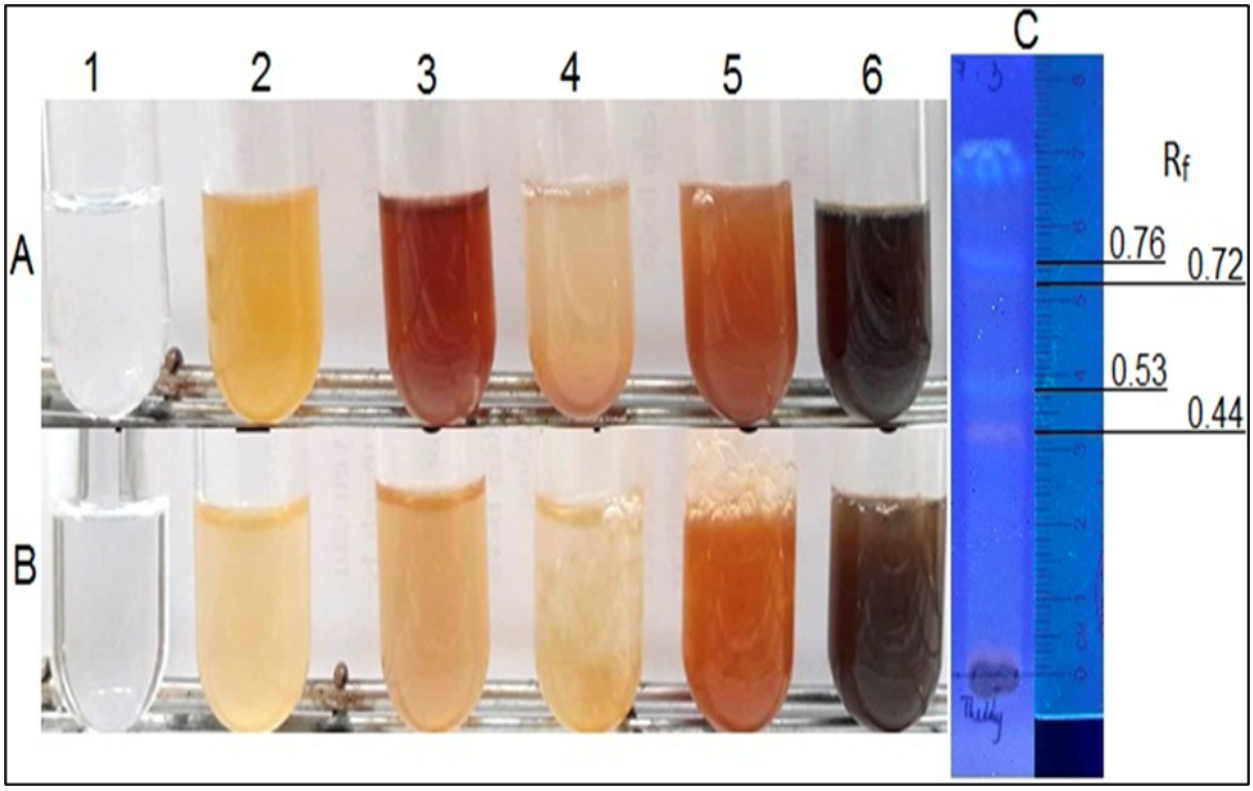

Figure 3: Quantitative determination of flavonoids by chemical reactions and TLC of *M. balbisiana* fruits

tochemicals is useful in predicting the existence of crude products and also valuable for the detection of phytoconstituents present in it. The presence in this medicinal herb of such essential phytochemical groups clearly indicates its therapeutic properties and validates to some its wide range of ethnomedicinal uses. Preliminary analysis results revealed the presence of compounds such as lipids, volatile oils, free triterpenoids, triterpenoid hydrolysis, proanthocyanidin, flavonoids, and saponins in *M. balbisiana* fruits (Table 1).

Extraction of crude extract and its fractions

Medicinal materials were extracted with 45% ethanol solvent at the concentration of 45% by the hot extraction method (ratio 1: 20). Results of extraction and extraction yields of crude extract and different fractions are presented in Figure 2.

Physicochemical parameters

The loss on drying, total ash and hydrochloric acid-insoluble ash values of dried materials and the crude extract of *M. balbisiana* fruits are given in Table 2. The proportion of the loss on drying of raw materials and the crude extract is 11.44% and 18.78%, respectively, which reached the standard according to regulations in Vietnamese Pharmacopoeia 5^{th} Edition (raw materials is less than 13% and the condensed extract is less than 20%). In addition, t he total ash content value is 6.72% for dried materials, and it is 12.78% for potential crude extract, which has been in accordance with the requirement of the quality of the raw materials (typically in the range of 4–12%, in some cases as high as 15–18%) and condensed extract ($\leq$35%) in compliance with the Vietnamese Pharmacopoeia 5^{th} Edition regulations. Whereas the percentage value of the hydrochloric acid-insoluble ash of *M . balbisiana* fruit raw materials is 0.60%, which has met the dried material level ($\leq$2.4%) as per the Vietnamese Pharmacopoeia 5^{th} Edition regulations.

Pharmacognostic standardizations have been carried out to establish the pharmacopeia standards, which are effective in identifying and authenticating the crude drugs obtained from their plant sources, including physicochemical analysis and phytochemical screening for a variety of medicinal plants (Partha and Rahaman, 2015). The loss on drying of the crude drug is a significant shelf-life parameter because insufficient drying promotes microorganisms that eventually undermine the biomass and the active principles of crude drugs. Therefore, it is directly linked to the stability and safety of crude medicines. Among the physical constants, the total ash content and hydrochloric acid-insoluble ash are also regarded as important parameters in appraising the purity and identity of a crude

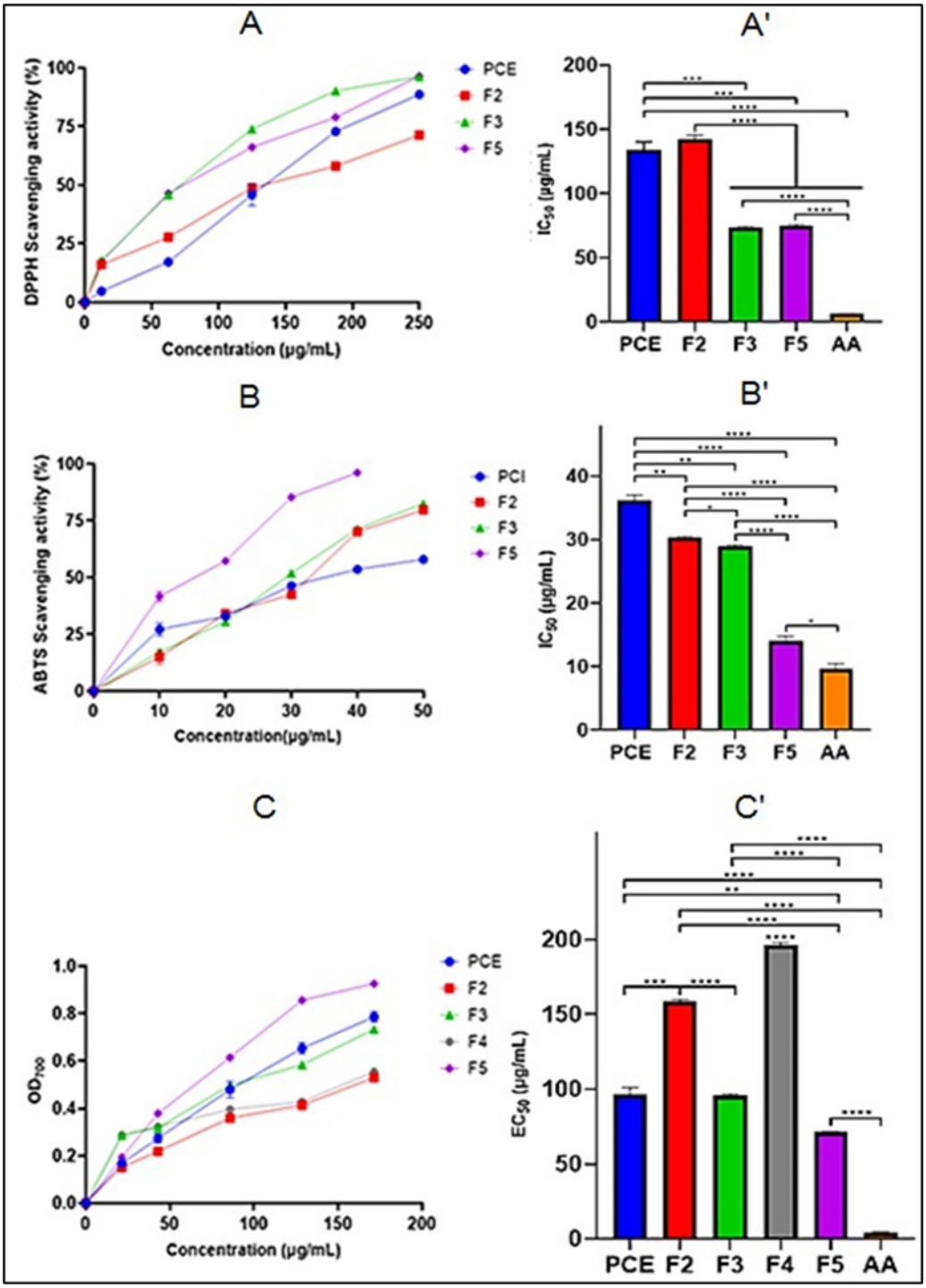

Figure 4: The antioxidant activity of potential crude extract and various fractions of *M. balbisiana* fruits by *in vitro* models

substance as well. It is considered as an indicator for mineral constituents of crude drugs or medicinal plants. It has now proven to be a very important factor in curing disease for phytochemicals in medicinal plants and bioactive phytochemicals provide the lead molecules for the development of novel drugs (Harborne and Williams, 1994). The results showed that raw materials and the potential crude extract from *M. balbisiana* fruits reached these criteria according to the general regulations of Vietnamese Pharmacopoeia 5^{th} Edition. Therefore, the potential crude extract from *M. balbisiana* fruits is qualified to carry out the next biological activity surveys and product development applications.

Quantitative determination of flavonoid using chemical reactions

The result showed that *M. balbisiana* fruit extract had a positive reaction with specific reagents of flavonoids (Figure 3A and Figure 3B). This revealed that *M. balbisiana* fruits contain compounds belonging to the flavonoids group. Therefore, *M. balbisiana* fruit extract was further analyzed by TLC.

Chemical reactions for the solution of raw materials (A) and solution of crude extract (B) and TLC chromatogram with R_f values (C). 1-Negative control (Ethanol 45%), 2- Positive control (Extract solution), 3- Reaction with NaOH 10% reagent, 4- Reaction with $Pb(CH_3COO)_2$ reagent, 5- Reaction with Cyanidin reagent, 6- Reaction with $FeCl_3$ 5% reagent.

The *M. balbisiana* fruit extract was prepared and detached by TLC. The successfully developed TLC plate produced different band colors and positions. The retention factor (R_f) value of each band was determined consiting of 0.44, 0.53, 0.72, and 0.76 (Figure 3C). The TLC profiling results showed the presence of phytochemicals within the *M. balbisiana* fruit extract.

The results of total polyphenol and flavonoid contents of *M. balbisiana* fruits by UV-Vis method are presented in Table 4. Comparing the results of total polyphenols content and total flavonoids content quantification using gallic acid and quercetin as a standard, respectively. The results showed that the total polyphenol content in extract was about 41.5 times higher than that of raw materials and the flavonoid content in extract was absolutely 9 times as much as the number of raw materials.

Evaluation of antioxidant activities potential crude and fractionated extracts

Many phytochemicals of plants are known to be responsible for the antioxidant property (Arunachalam and Parimelazhagan, 2014). The plant extracts have antioxidant activity that may be believed of containing compounds which have the ability to give hydrogen to free radicals. Hydrogen donors may be groups of polyphenol compounds (Sharma *et al.*, 2012) and flavonoids (Fidrianny *et al.*, 2015). Flavonoids can belong to polyphenol compounds. Flavonoids that unsubstituted OH-groups were not phenolic compounds, and the presence of OH-groups in a compound may cause the compound to become more polar. In a broad spectrum of pathological forms, free radicals are known to play a confirmatory function. Antioxidants allow the immune system to fight off and defend itself against these free radicals by either quenching free radicals or by guarding the defense workings (Umamaheswari and Chatterjee, 2008). It is a fact that a single assay in a living system cannot reflect the action of free radicals and antioxidants. Therefore, in the present study, we performed an array of assays including DPPH, ABTS and RP models to examine the oxidant scavenging potential of crude extracts. These assays could provide a more accurate description of antioxidant activity, which indicates that multiple antioxidant mechanisms might be responsible for the pharmacological effects of the extracts.

Generally, antioxidants can be classified into two main groups, including primary antioxidants and secondary antioxidants. DPPH assay is often applied in assessing primary antioxidants in plants where these respond to scavenge free radical from DPPH solution, thereby suppresses the development of the free radical initiation chain and by donating hydrogen atoms or electrons, prevent the propagation chain so that a free radical can be converted into a more stable form of products (Nurliyana *et al.*, 2010; Yan *et al.*, 2006). DPPH assay is a ubiquitously applied method to screen and evaluate the antioxidant property of plant extracts (Nanjo *et al.*, 1996). DPPH compound scavenging operation acts as an antioxidant in the extract by changing the color of the DPPH solution (Li *et al.*, 2011). The potential of antioxidant activity can be shown with IC_{50} value, which is the concentration of samples that can inhibit 50% of free radical DPPH activity. The lowest value of IC_{50} is indicated the highest antioxidant activity. The results showed that crude extract and various fractions of *M. balbisiana* fruits had DPPH radical quenching activity. Our data presented that the crude extract (PCE) displayed DPPH radical quenching property with an inhibition percentage >50% at 187.5 μg/mL with an IC_{50} value of 133.6 μg/mL. From Figure 4, The rate of DPPH radical scavenging (A), and IC_{50} values (A'); the rate of ABTS radical scavenging (B), and IC_{50} values (B'); the rate of reducing antioxidant power

(C), and EC_{50} values (C').(PCE) Crude extract, (F1) n-hexane, (F2) Chloroform, (F3) Ethyl acetate, (F4) n-butanol, and (F5) aqueous fractions, (AA) Ascorbic acid.Mean ± SEM (n=3). $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; and $^{****}p<0.0001$ significant difference compared with other extracts. At the highest concentration (250 μg/mL), the rate of the DPPH radical scavenging activities of different fractionated extracts were found around 71.26 to 96.51% (Figure 4A) with IC_{50} value of 143.2 μg/mL (F2 fraction), 73.57 μg/mL (F3 fraction), and 74.75 μg/mL (F5 fraction). Meanwhile, F1 and F4 fractions did not exhibit antioxidant activity by the mechanism of DPPH radical scavenging; hence, they were not determined IC_{50} value (Figure 4A').

Similarly, ABTS radical scavenging activity quantification is frequently used to appreciate the antioxidant proficiency *in vitro*. The PCE extract also exhibited antioxidant activity by the mechanism of ABTS radical scavenging with an inhibition percentage >50% at 40 μg/mL with an IC_{50} value of 36.25 μg/mL. The ABTS scavenging percentages for F2, F3, and F5 fractions were 14.98 - 79.80%, 17.19 - 84.11%, and 41.65 - 96.07% (Figure 4B), respectively. In other words, the F5 fraction had the best capacity in ABTS scavenging activity, followed by F3 and F2 fractions. The IC_{50} values of ABTS scavenging activity of fractionated extracts were determined (Figure 4B'). The percetage of the ABTS quenching activities were detected around 14.05 to 30.30 μg/mL with the order of F2 (30.30 μg/mL) > F3 (28.88 μg/mL) > F5 (14.05 μg/mL). F1 and F4 fractions did not exhibit antioxidant activity by the mechanism of ABTS radical scavenging hence, they were not determined IC_{50} value. The lowest IC_{50} shows the highest antioxidant potential since this value reflects the antioxidant concentration necessary to quench the free radical ABTS by 50% of the initial concentration. Sample which had very strong antioxidant activity when IC_{50} was lower than 50 μg/mL, strong antioxidant activity when IC_{50} was 50-100 μg/mL, medium antioxidant activity when IC_{50} was 101-150 μg/mL, while this value was greater than 150 μg/mL indicated a weak antioxidant (Blois, 1958). From the results, it may be identified that crude extract and some of the different fractions of *M. balbisiana* fruit were very strong antioxidants.

Evaluation of reducing antioxidant power (RP) assay is simple and widely applied to evaluate the efficacy of antioxidant substances in herbs to compete with the RP reagent and reduce the ferric to ferrous. Antioxidant compounds with this property are partaked as secondary antioxidants in which they inhibit the radical formation and interfere with oxidative damage. Besides, RP assay is also based on the antioxidants capability to reduce ferric (III) ions to ferrous (II) ions (Fu *et al.*, 2010). The RP assay determines the antioxidant capacity, which is the reducing activity of the compound joined in electron transfer reaction (Ediriweera and Ratnasooriya, 2009). Higher RP value also specifies higher antioxidant activity. Besides, the antioxidant potential can be also illustrated with EC_{50} values. EC_{50} is the concentration of samples that give half-maximal response – the 50% effective concentration. The lowest value of EC_{50} is indicated the highest antioxidant activity. Results showed that in the direction of increasing concentration, the optical density value of OD of crude extract and its fractions increases gradually (Figure 4C). The EC_{50} values of fractionated extracts were determined with the order of F5 (71.03 μg/mL) > F3 (96.07 μg/mL) > PCE (96.71 μg/mL) > F2 (158.91 μg/mL) > F4 (196.58 μg/mL) (Figure 4C'). Thus, the reducing antioxidant power of the aqueous fraction (F5) is the highest. F1 fraction did not exhibit reducing antioxidant power hence, they were not determined EC_{50} value.

In this study, the antioxidant activity was detected in crude extract and its fractions of *M. balbisiana* fruit which could react with free radicals to produce more stable forms and prevent free radical chain reactions. The results of this study, similar to some previous publications, also attested that some extracts and compounds isolated from *M. balbisiana* fruit have the antioxidant property (Revadigar *et al.*, 2017; Basumatary and Nath, 2018; Baskar *et al.*, 2011).

CONCLUSION

The raw materials and crude extract of *M. balbisiana* fruits were standardized based on Vietnamese Pharmacopeia 5^{th} Edition and the results satisfied the requirement about the loss on drying, total ash, and acid-insoluble ash. They were also investigated phytochemicals, microbiological characteristics of raw powders, qualitative by chemical reactions and thin layer chromatography. *M. balbisiana* fruit extract has high content of total polyphenols content and total flavonoids content. This study warranted that the crude extract and fractionated extracts had the properties of antioxidant. Therefore, *M. balbisiana* fruits may be incorporated as a functional dietary ingredient and useful as therapeutic agents in the treatment of free radical related pathological damages. Nevertheless, to explore the beneficial effect in human health, more biological studies, chemical compositions, and elucidations of the structure are needed in further investigations.

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Conflict of Interest

All the authors of this manuscript affirm that there is no conflict of interest.

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