Can Tho University Journal of Science
website: sj.ctu.edu.vn

DOI: 10.22144/ctu.jen.2020.002

Determination of total polyphenol, saponin contents, antioxidant and antibacterial activities of *Melastoma malabathricum* leaves by liquid-liquid extraction

Huynh Thanh Duy, Nguyen Van Thanh, Le Nhu Thuy, Nguyen Trieu Nhat Uyen and Nguyen Duc Do*

Biotechnology Research and Development Institute, Can Tho University, Vietnam

**Correspondence: Nguyen Duc Do (email: nddo@ctu.edu.vn)*

Article info.

Received 08 Jul 2019
Revised 04 May 2020
Accepted 31 Mar 2020

Keywords

Antibacterial, antioxidant, fraction, Melastoma malabathricum, polyphenol, saponin

ABSTRACT

The present study is aimed to investigate the total polyphenol and saponin contents, antioxidant and antibacterial activities of Melastoma malabathricum leaves extracts. These fractions were carried out using n-hexane, n-hexane:ethyl acetate (ratio 1:1), ethyl acetate and methanol solvents. The crude extract (CE) exhibited the highest amount of total polyphenol content by using the Folin-Ciocalteu assay. The n-hexane fraction (F1) showed the highest total saponin content which was determined by vanillin/H_2SO_4 method. The antioxidant activity of extracts was evaluated by using three different methods including DPPH radical scavenging assay, hydrogen peroxide assay (H_2O_2) and reducing power assay (Fe^{3+}). The results showed that the activity of the methanol fraction (F4) against DPPH was the strongest, the CE extract was the highest reducing power and the ethyl acetate fraction (F3) illustrated the strongest antioxidant capacity through H_2O_2 assay. Furthermore, all extracts were also tested for the antibacterial activity by agar well diffusion method at a concentration of 100 mg/mL. F4 showed the highest activity against Escherichia coli and Staphylococcus aureus, while there were no significant differences among all extracts when testing with Lactobacillus acidophilus. These findings provide important evidence that there is a correlation between the polyphenol content and antioxidant capacity and antibacterial activity. Besides, the saponin content was no contribution to antioxidant and antibacterial abilities.

Cited as: Duy, H.T., Thanh, N.V., Thuy, L.N., Uyen, N.T.N and Do, N.D., 2020. Determination of total polyphenol, saponin contents, antioxidant and antibacterial activities of *Melastoma malabathricum* leaves by liquid-liquid extraction. Can Tho University Journal of Science. 12(1): 8-15.

1 INTRODUCTION

Plants derived natural products are the source of most active components of medications, which in turn play a significant role in the treatment or prevention of human illnesses. The tropical plants have been investigated intensively during the last decades in order to evaluate the possibility of developing new, sustainable, natural and affordable cosmetics and drugs (Alnajar *et al*., 2012).

Many antioxidant compounds obtained from plant sources have been identified as free radical or active oxygen scavengers. In recent times, therefore interest has increased significantly in finding naturally occurring antioxidants for use in food or medicinal substances to replace the synthetic antioxidants.

Plant constituents, namely flavonoid and phenolic compounds and ginsenosides are broadly distributed and have been reported to exert multiple biological effects including antioxidant, anti-inflammatory, anticarcinogenic and anticancer activities (Sharma *et al*., 2011; Lu *et al*., 2009).

Melastomataceae plants originate in the tropic and subtropical regions, with a total of more than 4,000 species in the world. In the Southeast Asian region alone, the genus *Melastoma* comprises 22 species, two subspecies, and three varieties. It is native to tropical and temperate Asia and the Pacific Islands. The plant is one of the most common weeds that grow wildly and abundantly throughout the tropics, especially in the moist areas, and can be found in the Indian Ocean Islands, throughout South and South-East Asia, China, Taiwan, Australia, and the South Pacific Ocean. Various scientific papers were published on pharmacological properties of *Melastoma malabathricum* (Mua), the detailed and careful analysis revealed that it exhibited promising an anti-inflammatory, antifungal and antioxidant (Joffry *et al*., 2012). In addition, the most recent research on *M. malabathricum* revealed that its bioactive constituents exhibited free radical scavenging, anti-inflammatory, antibacterial and antiviral activities (Alnajar *et al*., 2012). The antibacterial and phytochemical screening of *Memecylon umbellatum* Burm leaves extract was shown to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* (Killedar *et al.*, 2012). Basing on the latest references, there are no such phytochemical reports concerning *M. malabathricum*, so the present study was designed to determine total polyphenol and saponin contents, antioxidant and bacterial inhibition property of extract of wild *Melastoma malabathricum* found in the Mekong Delta of Vietnam.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant materials

The healthy *M. malabathricum* leaves, which were not damaged by disease, insects or mechanical injury, were collected in the early morning from Hung Phu, Cai Rang district, Can Tho city.

2.1.2 Microorganisms

Microorganisms used in this study were both Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*L. acidophilus* and *S. aureus*). All the stock cultures were obtained from the Molecular Biology lab, Biotechnology Research and Development Institute, Can Tho University, Vietnam. Lysogeny broth (LB) was used as the media for the culturing of bacterial strains.

2.1.3 Chemicals

For natural compounds extraction, these chemicals were used ethanol (Vietnam), n-hexane (Vietnam), ethyl acetate (Vietnam), methanol (Vietnam), distilled water, Na_2SO_4 anhydrous (China). Testing for antioxidant activity used $FeCl_3.6H_2O$ (China), H_2SO_4 (China), gallic acid (China), Folin – Ciocalteu (Germany), Na_2CO_3 (China), H_2O_2 30% (China), $NaH_2PO_4.2H_2O$ (China), $Na_2HPO_4.12H_2O$ (China), $K_3[Fe(CN)_6]$ (China), CCl_3COOH (China), 2,2-diphenyl-1-picrylhydrazyl (DPPH)(US), ascorbic acid (China). Antibacterial tests used peptone (India), yeast extracts (Germany).

2.2 Methods

2.2.1 Fractionation procedure

A total of 700 g of dried *M. malabathricum* leaves were ground with a blender and extracted with 3.5 L ethanol 96% and combined with ultrasonic at 120W within 60 minutes. The extract was filtrated with Na_2SO_4 anhydrous and then evaporated in a rotary evaporator under reduced pressure, collected 50 g crude extract of *M. malabathricum* (CE). Next, 20 g of CE was dissolved with 50 mL of distilled water before extracting with of n-hexane (F1), n-hexane:ethyl acetate (1:1) (F2), ethyl acetate (F3) and the residue (F4) which was mixed with methanol, respectively by using liquid/liquid extraction. All solutions were rotated and vacuumed until the solvent has evaporated. Finally, the factions were stored at -4^{o}C.

2.2.2 Determination of total polyphenol content (TPC)

The TPC was estimated according to the procedure of Yadav and Agarwala (2011) with a few modifications. Briefly, the reaction mixture contained 0.1 mL of plant extract with 1 mL of Folin – Ciocalteu reagent and 1 mL of 2% solution Na_2CO_3 were then added. The absorbance was measured at 765 nm after 45 minutes of incubation at room temperature. Gallic acid was used as standard with concentration ranging from 20 to 120 µg/mL (blank sample was methanol). All the samples were measured in triplicate. The results were expressed as gallic acid equivalent (mg GAE/g of extracted compound) and determined from standard curve (y = ax + b) of gallic acid.

Total polyphenol content: $C = c.V/m$

Where, C: total polyphenol content (mg GAE/g of extracted compound); c: x value from gallic acid standard curve (µg/mL); V: volume of samples (mL); m: mass of samples in V (g).

2.2.3 Determination of total saponin content (TSC)

The TSC was estimated according to the procedure of Hiai *et al.* (1976) with a few modifications. 0.5 mL of samples was mixed with 0.2 mL of 4% (w/v) vanillin reagent and then 1.8 mL of 70% (v/v) sulfuric acid was added. After that, the mixture in test tubes was shaken before incubating at 60ºC for 10 minutes in a water bath, then for color development cooled in an ice-water bath for 15 minutes. Blank sample was mixtures of vanillin solution and sulfuric acid. The absorbance was measured at 550 nm. Ginsenoside (Rb1, Rg1, Rg3) was used as standard with concentration ranging from 10 to 60 µg/mL. All the samples were measured in triplicate. Result was expressed as ginsenoside (Rb1, Rg1, Rg3) equivalent (mg/g of extracted compound) and determined from standard curve (y = ax + b) of ginsenoside (Rb1, Rg1, Rg3).

Total saponin content: $S = c.V/m$

Where, S: total saponin content (mg/g of extracted compound); c: x value from saponin (Rb1, Rg1, Rg3) standard curve (µg/mL); V: volume of samples (mL); m: mass of samples in V (g).

2.2.4 DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay

The free radical scavenging activity was determined by the DPPH assay described by Blois (1958) with a few modifications. Briefly, 1.5 mL of the extract or ascorbic acid (control sample) at different concentrations in methanol was added to 0.5 mL of 0.1 mM DPPH solution into test tubes. The mixture was then incubated in darkness at room temperature for 30 minutes, and its absorbance pouring into a cuvette was measured at 517 nm. Ascorbic acid was used as a control sample with concentration ranging from 0.5 to 2.5 µg/mL. Blank sample was a mixture of methanol and DPPH solution. All the samples were measured in triplicate. The percentage of the DPPH radical scavenging was calculated as the following equation:

% scavenging of DPPH free radicals = $[(A_o - A)/A_o].100$

Where, A_o: the absorbance of blank sample; A: the absorbance of extracts or ascorbic acid.

The standard curve (y = ax + b) of ascorbic acid or extracts was established from percentage inhibition at its different concentrations. Then, the IC_{50} value of extracts or ascorbic acid was calculated.

2.2.5 Hydrogen peroxide (H_2O_2) scavenging assay

The ability of plant extracts to scavenge hydrogen peroxide can be estimated according to the method of Sharma *et al.* (2011). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (0.05 M pH 7.4). Briefly, 0.5 mL of the extracts or ascorbic acid (control sample) at different concentrations in phosphate buffer was added to 2.5 mL of hydrogen peroxide into test tubes. After 10 minutes, the absorbance was measured at 230 nm against a blank sample containing samples in phosphate buffer without hydrogen peroxide. Ascorbic acid was used as a control sample with concentration ranging from 50 to 100 µg/mL. All the samples were measured in triplicate. The percentage of hydrogen peroxide scavenging was calculated as the following equation:

% scavenging of H_2O_2 free radicals = $[(A_o - A)/A_o].100$

Where, A_o: the absorbance of blank sample; A: the absorbance of extracts or ascorbic acid.

The standard curve (y = ax + b) of ascorbic acid or extracts was established from percentage inhibition at its different concentrations. Then, the IC_{50} value of extracts or ascorbic acid was calculated.

2.2.6 Reducing power assay (Fe^{3+})

The method was based on the principle of increase in the absorbance of reaction mixtures that indicated the power of the samples and was described by Singhal *et al.* (2014). Briefly, 90 µL of a test sample solution in distilled water, 225 µL of 0.2M phosphate buffer (pH 6.6) and 225 µL of 1% (w/v) potassium ferricyanide were added into test tubes. The resulting mixture was incubated at 50ºC for 20 minutes, followed by the addition of 225 µL of trichloroacetic acid (10% w/v), 2125 µL of deionized water and 125 µL of ferric chloride solution (0.1% w/v), respectively. The absorbance was then measured at 700 nm against a blank sample (without a test sample solution). Ascorbic acid was used as a control sample with concentration ranging from 1.5 to 3.5 µg/mL. All the samples were measured in triplicate. The percentage of reducing power was calculated as the following equation:

% scavenging of Fe^{3+} = $[(A - A_o)/A_o].100$

Where, A_o: the absorbance of blank sample; A: the absorbance of extracts or ascorbic acid.

The standard curve (y = ax + b) of ascorbic acid or extracts was established from percentage inhibition at its different concentrations. Then, the IC_{50} value of samples or ascorbic acid was calculated.

2.2.7 Antibacterial activity

Antibacterial activity was determined by using the agar well diffusion method (Balouiri *et al*., 2016). All fractions and crude extracts were prepared in dimethyl sulfoxide (DMSO) and 100 mg/mL of each was used for activity, DMSO was used as negative control and ampicillin 0.5 mg/mL was used as control sample for *E. coli* and *S. aureus*, and ampicillin 0.05 mg/mL as the control sample for *L. acidophilus*. The LB agar plates were uniformly smeared with the suspension of bacteria. Wells (6 mm diameter) were created, to which 20 µL of different samples were loaded into each well. The plates were incubated at 37°C for 24 hours, after that the test materials having antibacterial activity inhibited the growth of microorganisms and a clear, distinct zone of inhibition was visualized surrounding the well.

2.3 Statistical analysis

The data were processed by Excel 2010 software and analyzed by Minitab (version 16), ANOVA analysis. The mean values were compared by the Tukey test. All the samples of each assay were measured in triplicate.

3 RESULTS AND DISCUSSIONS

3.1 Determination of TPC and TSC

The total polyphenol content was highest in CE (430 mg GAE/g extract), which was no significant difference with F4 (420 mg GAE/g extract) and F3 (344 mg GAE/g extract). F1 (97.5 mg GAE/g extract) was the lowest total polyphenol content. No significant differences were found between F1 and F2 (128 mg GAE/g extract). The solvent with higher polarity would extract a higher amount of total polyphenol content from *M. malabathricum* leaves. Table 1 exhibited the results of total polyphenol and saponin contents of crude extract and fractions from *M. malabathricum* leaves.

Polar solvent extract (methanol extract) from *M. malabathricum* leaves contained higher TPC than non-polar solvent extract (chloroform extract) (Zakaria *et al*., 2011). According to Handique and Gogoi (2016), the dry powders of *M. malabathricum* leaves were extracted in n-hexane, ethyl acetate and methanol by using Soxhlet extraction. The results were a similar trend to this study that the methanol extract contained the highest TPC with 9.6 mg GAE/g extract while the lowest was found in n-hexane extract (0.798 mg GAE/g extract). The TPC of all extracts in the present study was many times higher than *M. malabathricum* extract of this report due to different extraction and quantification methods were employed.

Table 1: TPC and TSC of crude extract and fractions of *M. malabathricum* leaves

Treatments	TPC (mg GAE/g extract)	TSC (mg/g extract)
CE	430.0±15.30^{a}	41.3±1.78^{d}
F1	97.5±6.56^{c}	95.2±1.98^{a}
F2	128.0±7.65^{c}	71.1±1.74^{b}
F3	344.0±2.85^{b}	58.5±2.90^{c}
F4	420.0±3.27^{ab}	32.6±2.00^{d}

Where: M. malabathricum crude extract (CE). n-hexane fraction (F1), n-hexane:ethyl acetate (1:1) fraction (F2), ethyl acetate fraction (F3) and the residue (F4). Values are expressed as mean ± standard deviation for triplicate. Values with the same superscripts in the same column are not significantly different at 99% level of confidence based on Tukey test (p<0.01). TPC was calculated based on the standard curve of gallic acid: y = 0.0036x – 0.0009, R^2 = 0.9919. TSC was calculated based on the standard curve of ginsenoside (Rb1, Rg1, Rg3): y = 0.0176x + 0.019; R^2 = 0.9934.

The results of the present study demonstrated that F1 was the highest saponin content (95.2 mg/g extract), which was almost three times higher than that of F4 (32.6 mg/g extract). There were no significant differences between CE (41.3 mg/g extract) and F4. The ginsenoside (Rb1, Rg1, Rg3) content was 9.36 mg/g when extracted six years old red ginseng roots with water solvent by using HPLC/UV (203 nm) (Lee *et al*., 2015). All extracts of this present study were significantly higher TSC than this report. In contrast to the TPC, the saponin (Rb1, Rg1, Rg3) content was extracted efficiently in non-polar and low polarity solvents (n-hexane and n-hexane:ethyl acetate:1:1). According to Kim *et al.* (2005), ginsenosides consist of aglycone and carbohydrates portions. The aglycone is the backbone of the ginsenosides with a hydrophobic four ring steroid-like structure that is non-polar, whereas the carbohydrates on carbons-3, 6 and 20 of the backbone are polar. Thus, ginsenosides are amphiphilic compounds and non-polar groups attended this reaction. Ginsenoside structures are first elucidated by

Shibata's group, and named as Rx according to their mobility on TLC plates, with polarity decreasing from index "a" to "h" (Nag *et al*., 2012). This report indicates that Rb1 compound is more polar than Rg1 and Rg3 so that the *M. malabathricum* extracts maybe contain a high amount of Rg1 and Rg3 compounds.

3.2 Determination of antioxidant activities

In the present study, the radical scavenging ability of the crude extract and fractions of *M. malabathricum* leaves was studied against DPPH, H_2O_2 and Fe^{3+}. The results were expressed by IC_{50} (the concentration of extract required to quench 50% of free radicals under the given experimental conditions). The extract with lower IC_{50} value has stronger antioxidant potential.

Based on the results shown in Table 2, IC_{50} was the lowest in F4 (1.61 µg/mL) which indicated that this extract had the strongest antioxidant ability to scavenge DPPH radical. No significant differences were found between ascorbic acid (1.52 µg/mL), CE (1.65 µg/mL) and F4. F1 was the highest IC_{50} value (8.29 µg/mL) and had the weakest antioxidant capacity. The *M. malabathricum* leaves were extracted with polar solvents showed higher antioxidant capacity than non-polar solvent. The methanol extract of *M. malabathricum* had strongest antioxidant capacity with IC_{50} value at 37.26 µg/mL while the n-hexane extract was the weakest (IC_{50} value at 314.51 µg/mL) compared to the IC_{50} value of Trolox (as a control) was 29.19 µg/mL (Handique and Gogoi, 2016). The results of the report above shows a similar trend with this study on the effect of solvents extraction on antioxidant activity.

Table 2: IC_{50} values (µg/mL) of ascorbic acid, crude extract and fractions of *M. malabathricum* in different assays

Treatments	IC_{50} values (µg/mL)		
	DPPH scavenging assay	Hydrogen peroxide (H_2O_2) scavenging assay	Reducing power (Fe^{3+}) assay
CE	1.65 ± 0.04^{d}	37.5 ± 0.15^{d}	3.42 ± 0.05^{c}
F1	8.29 ± 0.16^{a}	50.0 ± 0.83^{b}	24.2 ± 1.26^{a}
F2	7.13 ± 0.20^{b}	41.2 ± 0.56^{c}	23.0 ± 2.71^{a}
F3	2.35 ± 0.05^{c}	33.5 ± 0.38^{e}	9.15 ± 0.18^{b}
F4	1.61 ± 0.01^{d}	35.6 ± 0.05^{ed}	4.10 ± 0.24^{c}
Ascorbic acid	1.52 ± 0.02^{d}	88.7 ± 1.22^{a}	2.14 ± 0.16^{c}

Where: M. malabathricum crude extract (CE). n-hexane fraction (F1), n-hexane:ethyl acetate (1:1) fraction (F2), ethyl acetate fraction (F3) and the residue (F4). IC_{50} values are expressed as mean ± standard deviation for triplicate. Values with the same superscripts in the same column are not significantly different at 99% level of confidence based on Tukey test ($p<0.01$).

In the H_2O_2 scavenging assay, all extracts had an antioxidant activity stronger than ascorbic acid. Particularly, IC_{50} value was the highest in F1 (50 µg/mL) which showed the weakest antioxidant capacity. F3 (IC_{50} value at 33.5 µg/mL) had the strongest H_2O_2 free radical scavenging, which was no significant difference with F4 (IC_{50} value at 35.6 µg/mL). No significant differences were found between CE (IC_{50} value at 37.5 µg/mL) and F4. The results also showed that *M. malabathricum* leaves were extracted with low polarity solvents had stronger antioxidant capacity than non-polar solvents and high polarity solvents. According to Ruskin *et al.* (2017), *Canthium coromandelicum* leaves were extracted with different solvents, namely chloroform, ethyl acetate and ethanol. The results showed a similar trend with this study on the peroxide scavenging capacity had the strongest in ethyl acetate extract.

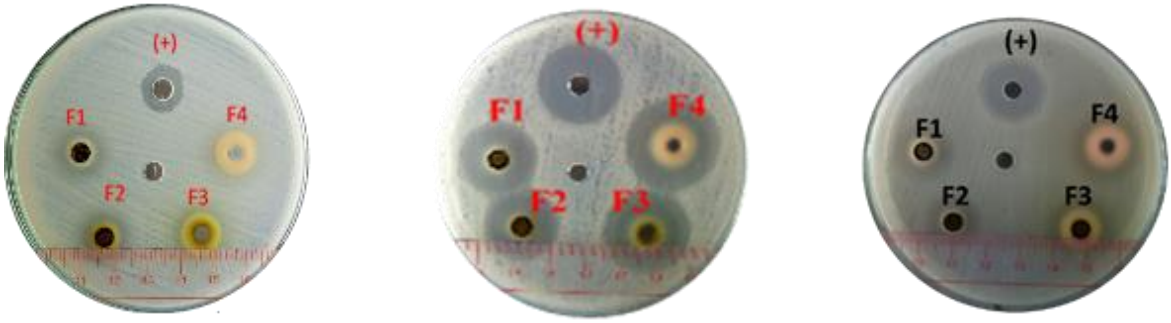
Based on the results of reducing power (Fe^{3+}) assay, the IC_{50} value was the highest in F1 (24.2 µg/mL) which was no significant difference with F2 (IC_{50} value at 23 µg/mL). F4 was the lowest in IC_{50} value (4.1 µg/mL) indicating that this extract had the strongest antioxidant capacity. No significant differences were found between CE (IC_{50} value at 3.42 µg/mL), F4 (IC_{50} value at 4.1 µg/mL) and ascorbic acid (IC_{50} value = 2.14 µg/mL). According to Sudan *et al*. (2014), fractions of *Arisaema jacquemontii* leaves were extracted with n-hexane, chloroform, ethyl acetate and methanol. The results demonstrated that the methanol fraction possessed the strongest reducing power with ferric reducing antioxidant power (FRAP) value of 1435.4 mmol/g dry weight, followed by the ethyl acetate fraction (1075 mmol/g dry weight), the chloroform fraction (300 mmol/g dry weight), while the n-hexane fraction

was the weakest (150 mmol/g dry weight). The results also showed a similar trend to the present study that samples were extracted with polar solvents had stronger a reducing power capacity than non-polar solvents.

Through three antioxidant assays, *M. malabathricum* leaves which were extracted with high polar solvents (ethanol and methanol), had the strongest antioxidant capacity in DPPH and reducing power assay, whereas the H_2O_2 scavenging activity was the strongest when the extract was fractionated by medium polar solvents (ethyl acetate). Polyphenol contents act as antioxidants because they have hydroxyl groups that can release protons in the form of hydrogen ions (Marjoni and Zulfisa, 2017). According to Hadique and Gogoi (2016), polyphenol contents are potential antioxidants and free radical-scavengers, hence there should be a close correlation between the content of polyphenol and antioxidant activity. Moreover, polyphenols are very valuable plant constituents in the scavenging of free radicals, due to their several phenolic hydroxyl groups. The amount of polyphenolic compound increases, antioxidant activity increases as well (Saha and Verma, 2016). The results of this study also showed a similar trend to the reports above on the total polyphenol content that is significantly correlated with antioxidant capacity. Indeed, CE and F4 contained a high amount of polyphenol content, so they had strong antioxidant activity. However, the TPC was not a correlation with H_2O_2 scavenging activity because the differences in H_2O_2 scavenging capacity among the extracts can be attributed to the structural features of their active components, which determine their electron-donating abilities (El-Chaghaby *et al*., 2014). In addition, different methods to measure antioxidant activity with various mechanisms may lead to different observations (Sun *et al*., 2005) so that the results of different antioxidant assays (DPPH, H_2O_2 and reducing power) were different. Furthermore, the antioxidant activity of a plant does not rely solely on phenolic compounds, but also on other substances such as carotenoids, vitamins and minerals (Tan *et al*., 2011). Besides, the TSC was not related to the antioxidant capacity (Table 1 and 2). According to Lee *et al*. (2016), antioxidant activity was not proportional to ginsenoside Rg1 content and significant correlation was observed. Considering ginsenoside's chemical structures, they are not electron-rich compounds like phenolic compounds, which are stabilized by the resonance delocalization of the unpaired electrons comprising the ring. Thus, ginsenosides are not easily prone to enter into efficient electron-donation reactions with oxidizing agents. Because of the weak degree of electron-donating ability of ginsenosides, they are probably poor radical scavengers in an antioxidant assay (Chae *et al*., 2010).

3.3 Antibacterial activity

The Figure 1 exhibited the results of the antibacterial activity of crude extract and frations from *M. malabathricum leaves*



(1) **(2)** **(3)**

Fig. 1: The antibacterial activity of fractions of *M. malabathricum* leaves against *E. coli* (1), *L. acidophilus* (2) and *S. aureus* (3) at the concentration in 100 mg/mL

All extracts from *M. malabathricum* leaves had activity against three testing bacteria (Table 3). CE and F4 exhibited the greatest inhibitory activity against *E. coli* and *S. aureus* which were not significantly different to those and ampicillin ($p<0.05$). The effectiveness against *E. coli*, *L. acidophilus* and *S. aureus* were arranged in descending order of CE, F4, F3, F2 and F1. The antimicrobial effect of all extracts against *L. acidophilus* were not significantly different (Fig. 1). The results also exhibited a significant positive correlation between the antibacterial effect and the polarity of solvent (all fractions were extracted in ascending order of the polarity of solvents).

Table 3: The antibacterial activity of fractions and crude extract of *M. malabathricum* against various bacteria in the concentration of 100 mg/mL after 24 hours of culture

Treatments	Inhibition zone diameter (mm)		
	Escherichia coli	***Lactobacillus acidophilus***	***Staphylococcus aureus***
CE	8.8±0.3[a]	13.0 ±0.0[b]	13.3±1.0[a]
F1	2.9±0.3[c]	11.7±0.6[b]	9.0±0.5[b]
F2	4.3±0.6[bc]	11.7±0.6[b]	10.0±0.1[ab]
F3	7.4±1.2[ab]	12.2±0.3[b]	11.7±0.6[ab]
F4	8.5±1.0[a]	12.3±0.6[b]	12.7±2.3[a]
Ampicillin	6.3±0.8[ab]	15.3±1.2[a]	12.0±1.0[ab]

Where: M. malabathricum crude extract (CE). n-hexane fraction (F1), n-hexane:ethyl acetate (1:1) fraction (F2), ethyl acetate fraction (F3) and the residue (F4). The diameters of inhibition zone values are expressed as mean ± standard deviation for triplicate. Values with the same superscripts in the same column are not significantly different at 95% level of confidence based on Tukey test (p<0.05). Ampicillin (0,5 mg/mL) was used as a control sample for E. coli and S. aureus, and ampicillin 0.05 mg/mL as a control sample for L. acidophilus.

The bacterial inhibitory ability of *M. malabathricum* extract was due to the compounds it contained. According to Cowan (1999), phytochemical compounds such as phenolics, tannins, flavonoids, alkaloids and quinone had the antimicrobial activity. In particular, the F4 and CE, which were extracted in high polarity solvents contained high polyphenol content (Table 1). The reason why polyphenol had the antibacterial activity as polyphenols were known as a factor that inactivated cellular enzymes or caused changes in membrane permeability (Moreno *et al*., 2006). In addition, phenolic compounds could form ligands with many metal ions such as ferric or cupric ions, which could cause iron deprivation in bacteria or formed hydrogen bonds with vital proteins such as microbial enzymes and thus inhibited many enzymes (Scalbert, 1991). Therefore, bacteria would be inhibited their growth and population. According to Killedar *et al.* (2012) using high polarity solvents such as ethanol and methanol exhibited potent inhibitory *E. coli* and *S. aureus* of the extracted from *Memecylon umbellatum* (Melastomaceae family) leaves, which was supported by the findings of this study. Another report of Alwash *et al*. (2013) detected kaempferol (Kf) compound in the methanol extract of *M. malabathricum* L. leaf. This compound inhibited *Staphylococcus* sp. with the value of the zone of the inhibition was 15.67 ± 0.58 mm and MIC value 0.25 mg/mL. Kaempferol is a natural flavonol found in many plants. Therefore, flavonoid compound present in *M. malabathricum* extract may be kaempferol and this compound has bioactive potential activity.

4 CONCLUSIONS

In this present study, the correlation between polyphenol content and antioxidant capacity and antimicrobial activity in solvents with different polarity and ratio were found. Besides, the saponin content might not be attributed to antioxidant and antibacterial activities. The crude extract of *M. malabathricum* is one promising source of the natural antioxidants as well as antimicrobial agents. Isolation and identification of active compounds in the crude extract are needed in future research which could be used for agriculture and pharmacy.

REFERENCES

Alnajar, Z.A.A., Abdulla, M.A., Ali, H.M., Alshawsh, M. A., and Hadi, A. H. A., 2012. Acute toxicity evaluation, antibacterial, antioxidant and immunomodulatory effects of *Melastoma malabathricum*. Molecules. 17(3): 3547-3559.

Alwash, M.S., Ibrahim, N., and Ahmad, W.Y.W., 2013. Identification and mode of action of antibacterial components from *Melastoma malabathricum* Linn leaves. American Journal of Infectious Diseases. 9(2): 46-58.

Balouiri, M., Sadiki, M., and Ibnsouda, S.K., 2016. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 6(2): 71-79.

Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature. 181: 1199–1200.

Chae, S., Kang, K.A., Youn, U., Park, J.S., and Hyun, J.W., 2010. A comparative study of the potential antioxidant activities of ginsenosides. Journal of Food Biochemistry. 34(s1): 31-43.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 12(4): 564-582.

El-Chaghaby, G.A., Ahmad, A.F., and Ramis, E.S., 2014. Evaluation of the antioxidant and antibacterial properties of various solvents extracts of *Annona squamosa* L. leaves. Arabian Journal of Chemistry. 7(2): 227-233.

Handique, J.G., and Gogoi, D., 2016. Antioxidant activities of the medicinal plants used for preparation of fermentation cakes of "Haanj", the rice based alcoholic beverage of Ahom community people of Assam, India. Int J Pharmacog Phytochem Res. 8(2): 217-222.

Hiai, S., Oura, H. and Nakajima T., 1976. Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. Planta Med. 29(2): 116-22.

Joffry, S.M., Yob, N.J., Rofiee, M.S., *et al*., 2012. *Melastoma malabathricum* (L.) Smith ethnomedicinal uses, chemical constituents, and pharmacological properties: a review. Evidence-Based Complementary and Alternative Medicine, 48 pages.

Killedar, S.G., and Harinath, N., 2012. Antimicrobial and phytochemical screening of different leaf extracts of *Memecylon umbellatum* Burm. International Research Journal of Pharmacy. 3(2): 188-192.

Kim, J.H., Hong, Y.H., Lee, J.H., *et al*., 2005. A role for the carbohydrate portion of saponin Rg3 in Na^+ channel inhibition. Molecules & Cells (Springer Science & Business Media BV). 19(1): 137-142.

Lee, J.W., Mo, E.J., Choi, J.E., *et al*., 2016. Effect of Korean Red Ginseng extraction conditions on antioxidant activity, extraction yield, and ginsenoside Rg1 and phenolic content: Optimization using response surface methodology. Journal of Ginseng Research. 40(3): 229-236.

Lee, S.M., Bae, B.S., Park, H.W., *et al*., 2015. Characterization of Korean Red Ginseng (*Panax ginseng* Meyer): history, preparation method, and chemical composition. Journal of Ginseng Research. 39(4): 384-391.

Lu, J.M., Yao, Q., and Chen, C., 2009. Ginseng compounds: an update on their molecular mechanisms and medical applications. Current Vascular Pharmacology. 7(3): 293-302.

Marjoni, M.R., and Zulfisa, A., 2017. Antioxidant activity of methanol extract/fractions of senggani leaves (*Melastoma candidum* D. Don). Pharmaceutica Analytica Acta. 8(557): 2-6.

Moreno, S., Scheyer, T., Romano, C.S., and Vojnov, A.A., 2006. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radical Research. 40(2): 223-231.

Nag, S.A., Qin, J., Wang, W., Wang, M.H., Wang, H., & Zhang, R., 2012. Ginsenosides as anticancer agents: in vitro and in vivo activities, structure-activity relationships, and molecular mechanisms of action. Frontiers in Pharmacology. 3: 25.

Ruskin, S.R., Vasanthakumari, B. and Citarasu, T., 2017. In vitro antioxidant activity of various leaf extracts of *Canthium coromandelicum* (Burm.f.) Alston. Asian Journal Pharmaceutical and Clinical Research. 10(5): 214-218.

Saha, S., and Verma, R.J. 2016. Antioxidant activity of polyphenolic extract of *Terminalia chebula Retzius* fruits. Journal of Taibah University for Science. 10(6): 805-812.

Scalbert, A., 1991. Antimicrobial properties of tannins. Phytochemistr., 30(12): 3875-3883.

Sharma, H.K., and Kumar, A., 2011. Evaluation of total phenol, flavonoid and in vitro antioxidant activity of methanolic extract of leaves of *Melastoma malabathricum* Linn. Asian Journal of Chemistry. 23(1): 434.

Singhal, M., Paul, A., and Singh, H.P., 2014. Synthesis and reducing power assay of methyl semicarbazone derivatives. Journal of Saudi Chemical Society. 18(2): 121-127.

Sudan, R., Bhagat, M., Gupta, S., Singh, J., and Koul, A., 2014. Iron (Fe II) chelation, ferric reducing antioxidant power, and immune modulating potential of *Arisaema jacquemontii* (Himalayan Cobra Lily). BioMed Research International. 2014: 7.

Sun, T., and Ho, C.T., 2005. Antioxidant activities of buckwheat extracts. Food Chemistry. 90(4): 743-749.

Tan, P.W., Tan, C.P., and Ho, C.W., 2011. Antioxidant properties: Effects of solid-to-solvent ratio on antioxidant compounds and capacities of Pegaga (*Centella asiatica).* International Food Research Journal. 18: 557-562.

Yadav, R.N.S., and Agarwala M., 2011. Phytochemical analysis of some medicinal plants. Journal of Phytology. 3(12): 10-14.

Zakaria, Z.A., Rofiee, M.S., Mohamed, A.M., Teh, L.K., and Salleh, M.Z., 2011. In vitro antiproliferative and antioxidant activities and total phenolic contents of the extracts of *Melastoma malabathricum* leaves. Journal of Acupuncture and Meridian Studies. 4(4): 248-256.