Food Research 8 (Supp. 4) : 57 - 64 (2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Evaluation of phytochemical, antioxidant and antimicrobial properties of different accessions of *Persicaria minor* (kesum)

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Article history:
Received: 16 August 2023
Received in revised form:16 August 2024
Accepted: 28 August 2024
Available Online: 20 September 2024

Keywords:
Bioactivities,
Herbs,
Methanol,
LC-MS,
Phenolics,
Profiling

DOI:
https://doi.org/10.26656/fr.2017.8(S4).6

Abstract

Persicaria minor (Huds.) Opiz. or locally known as *kesum* is an aromatic medicinal plant from the family Polygonaceae. It is originated from Southeast Asian countries such as Indonesia, Vietnam, Thailand and Malaysia. The plant leaf has many claims with regards to its traditional uses, but is popularly consumed as fresh vegetable (*ulam*), flavouring agent and food additive. Previous research showed that *P. minor* possessed antioxidant and antimicrobial activities. However, information on the effect of antioxidant and antimicrobial activities of different accessions of *P. minor* is still lacking. Therefore, the current work was undertaken to evaluate six different accessions of *P. minor* comprising MKSM002, MKSM004, MKSM006, MKSM011, MKSM013 and MKSM020. These accessions were obtained from various locations in Peninsular Malaysia and established in MARDI Headquarters herbal germplasm. The aerial parts of the plants were dried under a hot air oven at 50°C prior to extraction. From the results, MKSM002 and MKSM006 methanolic extracts were the most potent antioxidant due to the strongest ferric reducing (13.19 µmol Fe/g) and DPPH radical scavenging (IC_{50} 0.39 mg/mL) activities, respectively. Antimicrobial activity was determined against common pathogenic bacteria i.e., *Bacillus cereus, Staphylococcus aureus, Cronobacter sakazakii* and *Enterobacter aerogenes* using disc diffusion assay. Among the extracts, MKSM002, MKSM004 and MKSM006 were the most effective, being able to significantly ($P<0.05$) inhibit the growth of most of the bacteria tested. The most active inhibition was recorded against *E. aerogenes* with the diameter of inhibition zones ranging from 14.12 mm to 15.75 mm. Phytochemical screening of the extracts showed that these extracts contained a substantial amount of total phenolics and total flavonoids. Liquid chromatography-tandem mass spectrometry-quadrupole time-of-flight (LC–MS-QTOF) analysis of the selected extracts revealed the presence of various phytochemicals, with flavonoids and their derivatives being the most predominant ones. In conclusion, the selected *P. minor* accessions can be promoted as a source of planting material source for breeding, conservation and commercial purposes. The findings are also important in the search for antioxidant supplementation and healthcare products in the future.

1. Introduction

Persicaria minor (Huds.) Opiz or previously known as *Polygonum minus* is an aromatic medicinal plant from the family Polygonaceae. It originated from Southeast Asian countries such as Indonesia, Vietnam, Thailand, and Malaysia (Christapher *et al.,* 2015; Lau *et al.,* 2020). Depending on the country, the common name of *P. minor* is interestingly varied. Its English name includes small water pepper, pygmy smartweeds, tear-thumb, slender persicaria and Vietnamese coriander. However, in Malaysia, this herb is locally known as kesum, cenohom or laksa leaf (Abdullah *et al.,* 2017). It has many claims with regards to its traditional uses, but is popularly consumed as culinary herb. The leaves can be eaten raw as ulam, equivalent to salad in other countries, and are often used as flavouring ingredient due to their strong aroma and fragrance (Vimala *et al.,* 2011). Traditionally, the leaves have been used to treat various ailments including indigestion, constipation, stomach disorders, skin fungal infection, dandruff, postnatal tonic, sprains and body aches (Vimala *et al.,* 2011; Abubakar *et al.,* 2015; Abdullah *et al.,* 2017).

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eISSN: 2550-2166
Published by Rynnye Lyan Resources

The excellent health benefits that have been reported from plants are mostly ascribed to bioactive non-nutritional chemical compounds commonly named phytochemicals. The diverse array of phytochemicals can be categorised on the basis of their biosynthetic origin which includes but is not limited to phenolics, alkaloids, steroids, terpenes and saponins (Chew *et al.,* 2011). The fragrant nature of *P. minor* leaves is attributed to its high content (72.54%) of volatile aromatic essential oils, which are mainly sesquiterpenes and aliphatic aldehydes. Approximately, 77 compounds have been identified by Gas Chromatography Mass Spectrometry (GC-MS) (Abdullah et al., 2015). In addition to the volatile compounds, phenolics and flavonoids have also been reported to be responsible for various pharmacological activities of *P. minor* which include antioxidant, anti-microbial, anti-cancer, immunostimulant (Christapher *et al.,* 2015), anti-diarrheal, anti-ulcer, anti-inflammatory (Abubakar *et al.,* 2015), anti-hyperlipidemic, anti-acetylcholinesterase and cytotoxicity (Abdullah *et al.,* 2015).

However, in Malaysia, *P. minor* has not been comprehensively investigated as compared to other herbal plants and industrial crops. Although traditional people have used *P. minor* a great deal in many different aspects of their lives, the present generation has less interest in utilizing it due to the lack of awareness and knowledge of their potential values. Therefore, screening of different accessions of *P. minor* is warranted as the initial step for identifying the best planting materials with high quality and potentials. The main objective of the current study was to determine (1) *P. minor* accessions with high antioxidant and antimicrobial activities, as well as (2) to identify the phytochemicals that contribute to the activities. The data obtained from the study may provide a greater insight into the potentials of different *P. minor* accessions, and serve as a guideline for further use of *P. minor* in disease prevention and health promotion, based on the active compounds and their biological attributes. This information is important in the search for good quality planting materials for breeding, conservation, product development and commercial purposes.

2. Materials and methods

2.1 Plant materials and preparation of extracts

A total of six *P. minor* accessions were collected from various local farms in Peninsular Malaysia, and established in MARDI Headquarters herbal germplasm under similar agronomic practices. The selected accessions were labelled as MKSM002, MKSM004, MKSM006, MKSM011, MKSM013, MKSM020 and used in this study. Upon arrival in the laboratory, they fresh aerial parts were washed with running tap water to remove surface pollutants and cut into small pieces. They were then dried under a hot air oven at temperature of 50°C for 48 hrs. After drying, the samples were ground into a fine powder (moisture content of 8-10% dry basis) and keep in air tight container prior to extraction. The samples were extracted with 70% methanol by soaking them in the respective solvents (1:10) with continuous stirring on an orbital shaker (Protech, Malaysia) set at 150 rpm, for approximately 24 hrs. Extraction was repeated three times under identical conditions. Following this, the filtrates were combined and brought to complete dryness using a rotary evaporator (Buchi, Switzerland) to obtain the crude extracts. The crude extracts were stored at 4°C till analyses.

2.2 Determination of antioxidant activity

2.2.1 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The scavenging activity of the test samples on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was evaluated according to our previous work (Mirfat *et al.,* 2020). Various concentrations of the extracts in methanol were prepared to give a final volume of 7 µL and were mixed with 280 µL of methanolic solution containing DPPH radicals resulting in a final concentration of 0.06 mM. The reaction mixture was vigorously shaken and left to stand for 30 mins in the dark. Ascorbic acid (vitamin C) was used as a positive control. The negative control contained all reagents without the test samples, while methanol was used as a blank. The DPPH radical scavenging activity was determined by measuring the absorbance at 517 nm using a microplate reader (Eon Biotek, VT, USA). The percentage of inhibition of the DPPH radicals was calculated as follows:

Inhibition (%) = (Absorbance of control − Absorbance of test sample) / (Absorbance control) x 100%

A graph of the DPPH inhibition percentage of each sample against the sample concentration was plotted. The final results were expressed as IC_{50} value, which is the inhibitory concentration at which DPPH radicals were scavenged by 50%. All procedures were conducted in triplicates with a minimum exposure to light.

2.2.2 Ferric reducing antioxidant power assay

Ferric reducing antioxidant power (FRAP) assay was determined based on the reduction of ferric-tripiridyltriazine (Fe^{3+}-TPTZ) to a blue-coloured ferrous form (Fe^{2+}-TPTZ) (Mirfat *et al.,* 2020). The working FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (Sigma, USA) solution and 20 mM $FeCl_3.6H_2O$ (Sigma, USA) in a ratio of

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10:1:1, prior to use and warmed at 37°C in water bath. A total of 7 µL of sample and 20 µL of distilled water were added to 200 µL of FRAP reagent and incubated at 37°C for 4 mins. The absorbance of the reaction mixture was measured at 593 nm against a blank. Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) was used as a reference standard (100-1000 mM) to produce a calibration curve. The final results were expressed as the concentration of antioxidants having a ferric-reducing ability in Fe µmol/ mg sample.

2.3 Determination of antimicrobial activity

The antimicrobial activity was determined using disc diffusion assay according to the National Committee for Clinical Laboratory Standards (NCSSL) (1999) and Kirby-Baeur methods (Mohd Effendi *et al.,* 2022). The pathogen strains used in this study consisted of positive Gram bacteria; *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 10876, and negative Gram bacteria; *Cronobacter sakazakii* ATCC 29544 and *Enterobacter aerugenes* ATCC 13048. All strains were grown in nutrient broth overnight before being diluted with Ringer's solution to a concentration of 1×10^8 CFU/ mL equivalent to a 0.5 McFarland standard turbidity. An aliquot of 100 µL of the strain inoculum was pipetted and spread on solidified Mueller Hinton agar in a petri dish using a sterile cotton swab, before placing a sterile diffusion disc. A total of 20 µL of the extract solution was pipetted onto the disc. Penicillin disc (10 µg) was used as a control. All petri dishes were incubated in an incubator for 24 hrs at 37°C. Any formation of clear zone (the zone of inhibition) around the disc was an indication of antimicrobial activity and the diameter was measured in mm.

2.4 Phytochemical screening

2.4.1 Determination of total phenolic content

Total phenolic content of the test samples was estimated according to the Folin–Ciocalteu colorimetric method as described in our previous work (Mirfat *et al.,* 2020). Briefly, a 50 µL test sample was mixed with 100 µL Folin Ciocalteu's phenol reagent. After 3 mins, 100 µL 10% Na_2CO_3 was added to the reaction mixture and allowed to stand in the dark for 60 mins. The analysis was carried out in triplicates with a minimum exposure of light. The resulting blue-coloured complex was measured at 725 nm against a blank. Gallic acid was used as a reference standard and the content of total phenol was expressed in gallic acid equivalents (GAE) in milligram per g samples through the calibration curve.

2.4.2 Determination of total flavonoid content

The aluminium chloride method was used for the determination of total flavonoids following a previously reported by Mirfat *et al.* (2020). An aliquot of 30 µL of extract was diluted with 120 µL dH_2O. Initially, 9 µL 5% $NaNO_2$ solution was added and allowed to react for 5 mins. Then, 9 µL 10% $AlCl_3$ solution was added and left to stand for 5 mins. Finally, 60 µL NaOH and 72 µL dH_2O were added, and the mixture was well-mixed with a vortex. All samples were analysed in triplicates and the absorbance was measured immediately at 510 nm against a blank. The total flavonoid content was calculated from the calibration curve using rutin as a standard reference. The data were expressed as rutin equivalents (RE) in milligram per g samples.

2.5 Identification of phenolic compounds using ultra-high-performance liquid chromatography-qudarupole time-of-flight mass spectrometry

Ultra-high pressure liquid chromatography (UHPLC) was performed on ACQUITY UPLC I-Class system from Waters (Milford, USA), consisting of a binary pump, a vacuum degasser, an auto-sampler and a column oven (Mirfat *et al.,* 2020). Compounds were chromatographically separated using a column ACQUITY UPLC HSS T3 (100 mm × 2.1 mm × 1.8 µm), maintained at 40°C. A linear binary gradient of water (0.1% formic acid) and acetonitrile was used as mobile phase A and B, respectively. The mobile phase composition was changed during the run as follows: 0 min, 1% B; 0.5 min, 1% B; 16.00 min, 35% B; 18.00 min, 100% B; 20.00 min, 1% B. The flow rate was set at 0.6 mL/min and the injection volume was 1 µL.

For MS characterisation, the UHPLC system was coupled to a Vion IMS quadrupole time-of-flight (QTOF) hybrid mass spectrometer (Waters) operated at 50,000 FWHM, and equipped with a Lock Spray ion source. The ion source was operated in negative electrospray ionisation (ESI) mode under the following specific conditions: capillary voltage, 1.50 kV; reference capillary voltage, 3.00 kV; source temperature, 120°C; desolvation gas temperature, 550°C; desolvation gas flow, 800 L/h, and cone gas flow, 50 L/h. Nitrogen (>99.5%) was employed as desolvation and cone gas. Data were acquired in high-definition MSE (HDMSE) mode in the range of m/z 50 - 1500 at 0.1 s/scan. Thus, two independent scans with different collision energies (CE) were alternatively acquired during the run: a low-energy (LE) scan at a fixed CE of 4 eV, and a high-energy (HE) scan where the CE was ramped from 10 to 40 eV. Argon (99.999%) was used as collision-induced-dissociation (CID) gas. The MS data were processed and analysed with UNIFI Software (Waters). All compounds were identified by comparing full scan mass spectra, parent ion and MS ions information with those in the database.

https://doi.org/10.26656/fr.2017.8(S4).6

2.6 Statistical analysis

All experiments were carried out in triplicates and presented as means ± standard deviations (SD). The data were statistically analysed by analysis of variance (ANOVA) and Tukey's post hoc test using SPSS software, version 20 (SPSS Inc; Chicago, IL, USA). A value of $P<0.05$ was considered to be statistically significant.

3. Results and discussion

Antioxidants have become scientifically interesting owing to their numerous health benefits. They help to protect the human body against oxidative damage by scavenging the harmful free radicals which have been associated with the beginning of many disorders, such as ageing, anaemia, arthritis, asthma, diabetes, hypertension, inflammation, myocardial infarction, atherosclerosis and neurodegenerative diseases (Krishnasamy and Muthusamy, 2015).

The DPPH radical scavenging assay measures the ability of antioxidants to quench DPPH radicals by providing hydrogen atoms or by electron donation conceivably via a free radical attack on the DPPH molecules. 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical is a stable molecule with an unpaired electron. In the presence of antioxidants, the unpaired electron of DPPH$^{\cdot}$ radical becomes paired to form a non-radical reduced form DPPH-H (Kassim *et al.,* 2013). Results of the DPPH radical scavenging activity of different *P. minor* accessions are presented in Table 1. The activities varied from 0.39 mg/mL to 29.36 mg/mL, with accession MKSM006 being the strongest (IC_{50} 0.39 mg/mL) ($P<0.05$). This is followed by MKSM011 (IC_{50} 0.51 mg/mL) which showed no significant difference between each other. Meanwhile, the lowest scavenging activity was observed in MKSM020 (IC_{50} 29.36 mg/mL) ($P<0.05$). These antioxidant activities are inversely proportional to the IC_{50} values, and values lower than 10 mg/mL are indicative of effective antioxidant activity (Lee *et al.,* 2007).

Another commonly used method to evaluate antioxidant activity is the FRAP assay. It measures the ability of the antioxidants contained in the extracts to reduce ferric-tripiridyltriazine (Fe^{3+}-TPTZ) complex to a blue-coloured ferrous-tripyridyltriazine (Fe^{2+}-TPTZ) at low pH. Fe^{3+} is an oxidising agent that accepts electrons and causes another reactant to be oxidised. Fe^{2+} which is produced from the reduction of Fe^{3+}, is a well-known pro -oxidant which is a reactive oxygen species (ROS) that can cause oxidative damage to lipids, proteins and nucleic acids, resulting in various pathologic events and/ or diseases (Benzie and Strain, 1996). Therefore, the ability of the antioxidants in reducing Fe^{3+} may reflect their ability in reducing reactive species. From the results (Table 1), a slightly different trend can be observed in the ferric reducing activity. Accession MKSM002 ranked first with 13.19 µmol Fe/g, followed by MKSM006 with 10.98 µmol Fe/g ($P<0.05$). Accession MKSM020 remained the weakest antioxidant as indicated by the lowest ferric reducing ability (3.51 µmol Fe/g) ($P<0.05$).

Table 1. Antioxidant activities of different accessions of *P. minor.*

Sample	DPPH radical scavenging activity, IC_{50} (mg/mL)	FRAP activity (µmol Fe/g)
MKSM002	5.368^{c}	13.194^{b}
MKSM004	17.288^{e}	5.149^{f}
MKSM006	0.388^{b}	10.978^{c}
MKSM011	0.505^{b}	8.154^{d}
MKSM013	10.839^{d}	6.506^{ef}
MKSM020	29.356^{f}	3.513^{g}
Vitamin C	0.061^{a}	17.422^{a}

Values are presented as means of triplicate determination (n = 3). Values with different superscripts within the same column are statistically significantly different at $P<0.05$. IC_{50} is the inhibitory concentration of sample to scavenge 50% DPPH radicals.

As denoted in many previous reports, *P. minor* was a potential source of antioxidants (Vimala *et al.,* 2011; Abdullah *et al.,* 2017; Lau *et al.,* 2020). Our results were consistent with Christapher *et al.* (2015) who reported that the methanolic extracts of *P. minor* exerted high antioxidant activity when assessed using DPPH radical scavenging and FRAP assays. The pronounced activity could be attributed to the presence of antioxidant compounds, i.e., polyphenols that include flavonoids, phenolic acid, tannins, and others (Christapher *et al.,* 2015).

Many aromatic and medicinal plants have been widely used as antimicrobial agents in an effort to mitigate the spread and rapid emergence of antimicrobial resistance. Antimicrobial resistance is a serious threat to human health globally. World Health Organisation (WHO) has identified *S. aureus* and *Enterobacter* species as among antibiotic-resistant priority pathogens that represent alarming threat to humans (León-Buitimea *et al.,* 2020). Therefore, in the present study, antimicrobial activity of *P. minor* was determined against common pathogenic bacteria which include *Bacillus cereus, Staphylococcus aureus, Cronobacter sakazakii* and *Enterobacter aerogenes.*

As shown in Table 2, MKSM002, MKSM004 and MKSM006 exhibited the most active inhibitions, particularly against *E. aerogenes,* with the diameter of

https://doi.org/10.26656/fr.2017.8(S4).6

inhibition zones ranging from 14.12 mm to 15.75 mm (P<0.05). There were no significant differences observed among them. Interestingly, the inhibition zones were significantly (P<0.05) higher than the commercial drug penicillin (10.00 mm), which clearly indicated the strong antimicrobial effect of *P. minor.* MKSM002, MKSM004 and MKSM006 were also effective against *S. aureus,* being able to significantly (P<0.05) inhibit the growth of the bacteria with inhibition zones of 11.00 mm, 11.75 mm and 10.75 mm, respectively. Among all the bacterial strains tested, *B. cereus* was found to be resistant to penicillin. Meanwhile, *P. minor* accessions showed weak to moderate inhibitory activities against *B. cereus* and *C. sakazakii.*

Table 2. Antimicrobial activities of different accessions of *P. minor.*

Sample	Diameter of inhibition zones (mm)			
	B. cereus	*S. aureus*	*C. sakazakii*	*E. aerogenes*
MKSM002	8.25^{fgh}	11.00^{cd}	7.50^{hi}	14.12^{b}
MKSM004	9.75^{def}	11.75^{c}	7.25^{i}	14.25^{b}
MKSM006	8.25^{fgh}	10.75^{cde}	8.25^{fgh}	15.75^{b}
MKSM011	9.50^{def}	9.75^{def}	9.25^{defg}	10.50^{cde}
MKSM013	8.75^{efgh}	9.75^{def}	9.00^{defg}	9.00^{defg}
MKSM020	8.50^{efgh}	8.50^{efgh}	9.50^{def}	8.25^{fgh}
Penicillin	0.0^{j}	40.0^{a}	11.00^{cd}	10.00^{cdef}

Values are presented as means of triplicate determination (n = 3). Values with different superscripts within the same column are statistically significantly different at P<0.05.

Our findings were in line with Abubakar *et al.* (2015) who reported that *P. minor* had strong antimicrobial activity against *S. aureus* and several other pathogenic bacterial strains. Many *S. aureus* strains are sensitive towards the aromatic properties of terpene compounds which cause severe damage to the cell membranes. The same author also described that the potent antibacterial activity of *P. minor* could be ascribed to the presence of tannins, flavonoids and terpenes. (Abubakar *et al.,* 2015).

There are a large number of different types of phytochemicals that might contribute to the antioxidant and antimicrobial activities of *P. minor.* To explore the influence of the phytochemicals on the biological activities, total phenolic content (TPC) and total flavonoid content (TFC) were determined. Phenolic compounds or polyphenols produced by the phenylpropanoid pathway constitute one of the most numerous and ubiquitously distributed groups of plant secondary metabolites (Passo Tsamo *et al.,* 2015). Flavonoids, phenolic acids and tannins are among the major classes of phenolics that have received great attention with regard to their various health benefits (Irondi *et al.,* 2015).

Results of TPC and TFC of *P. minor* accessions are presented in Table 3. Significant variations of the phytochemical contents were observed among the extracts. This could be due to genetic factors of the accessions. The highest amount of total phenolic were recorded in MKSM006 (83.79 mg GAE/g), followed by MKSM002 (74.48 mg GAE/g) with no significant difference between them. Meanwhile, the lowest TPC corresponded to MKSM020 (22.53 mg GAE/g) (P<0.05). Similarly, the amount of total flavonoid was also in the order of MKSM006 (33.91 mg RE/g) > MKSM002 (25.67 mg RE/g) > MKSM020 (10.53 mg RE/g). The findings indicated that phenolics and flavonoids may be responsible for the pronounced antioxidant and antimicrobial activities of *P. minor.*

Table 3. Total phenolic and total flavonoid contents of different accessions of *P. minor.*

Sample	Total phenolic content (mg GAE/g)	Total flavonoid content (mg RE/g)
MKSM002	74.484^{ab}	25.660^{bc}
MKSM004	42.345^{d}	19.841^{cd}
MKSM006	83.795^{a}	33.908^{a}
MKSM011	58.123^{c}	21.920^{bcd}
MKSM013	45.706^{d}	21.255^{bcd}
MKSM020	22.533^{e}	10.526^{e}

Values are presented as means of triplicate determination (n = 3). Values with different superscripts within the same column are statistically significantly different at P<0.05.

Our results were also in agreement with Barchan *et al.* (2014) who reported that phenolic compounds of a plant extract as measured by TPC and TFC were well-correlated with antioxidant activity. The compounds have been identified as primary antioxidants and popular dietary supplements (Kala *et al.,* 2016), and hence exhibit a wide range of biological effects (Kua *et al.,* 2016). Besides their established antioxidant activity, phenolic compounds also exhibit antimicrobial activity against a broad spectrum of bacteria. Flavonoids and phenolic acids mediate their antibacterial mechanisms through (1) inhibition of bacterial virulence factors such as enzymes and toxins, (2) interaction with cytoplasmic membrane (3) suppression of biofilm formation and (4) synergistic effect with antibiotics (Miklasińska-Majdanik *et al.,* 2018).

On the basis of their promising antioxidant and antimicrobial activities, MKSM004 and MKSM006 were further analysed using liquid chromatography-tandem mass spectrometry-quadrupole time-of-flight (LC–MS-QTOF) to identify the major putative compounds. Figure 1 depicts the base peak chromatogram obtained from LC -MS/MS analysis of *P. minor* accession MKSM004 and MKSM006. The compounds were tentatively identified

https://doi.org/10.26656/fr.2017.8(S4).6

by interpreting their deprotonated parent molecular ions $[M-H]^-$ and by comparison with previous literature and reference data from the related databases.

The putative identifications of the major compounds are presented in Table 4, with flavonoids and derivatives notably predominated in the extracts. The analysis revealed that flavonoids were represented mostly by flavonols, derived from the aglycones; kaempferol and quercetin derivatives. The most predominant compounds were tentatively identified as 6-hydroxykaempferol-3-O-glucoside, tubuloside E, cimicifugic acid B and tectorigenin. Other detected compounds in the accessions with previously reported antioxidant and antimicrobial activities include kaempferol-3-O-β-D-glucopyranoside, quercetin-3-O-β-D-glucuronide and epicatechin-3-O-gallate, among others.

Quercetin has been reported to possess antioxidant activity which acts as an anti-inflammatory, anti-histamine, anti-viral and anti-cancer (Anand David *et al.,* 2016). Its derivative, quercetin-3-O-β-D-glucuronide was shown to be highly potent in enhancing mood, cognitive function and life improvement for middle-aged women when consuming supplements containing the active compound (Yahya *et al.,* 2017).

4. Conclusion

Two *P. minor* accessions; MKSM004 and MKSM006 can be suggested as the most promising natural antioxidant and antimicrobial agents. Therefore,

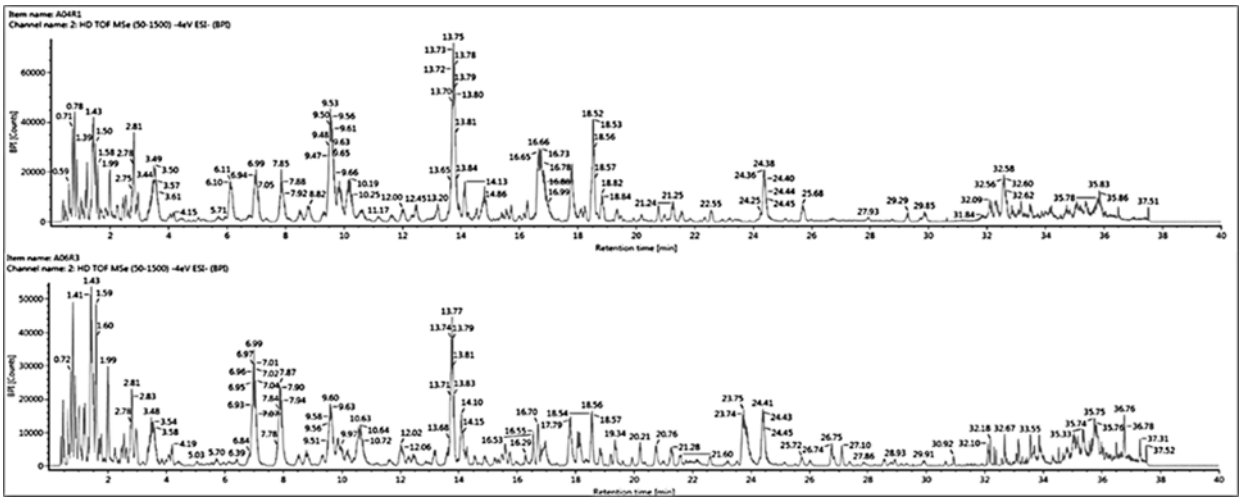

Figure 1. LC-MS base peak chromatogram of the major compounds of *P. minor* accession (a) MKSM004 and (b) MKSM006 under negative ion mode.

Table 4. LC-MS profile of MKSM004 and MKSM006 methanolic extract under negative ion mode.

RT (min)	Proposed compound	Error (ppm)	Molecular mass	MS [M-H] (m/z)
Major compounds				
MKSM004				
6.12	6-Hydroxykaempferol-3-O-glucoside	0.8	464.0959	463.0886
6.38	Tubuloside E	-2.1	650.2211	649.2124
16.66	ω-Hydroxyemodin	-1.2	286.0477	285.0401
28.75	3β-Hydroxypterondontic acid	-3.2	250.1569	249.1488
MKSM006				
10.61	Cimicifugic acid B	0.3	448.1006	447.0934
23.73	Tectorigenin	-1.3	300.0634	299.0557
Other compounds				
1.5	Arecatannin A1	3.8	866.2058	865.2019
6.97	Epicatechin gallate (Epicatechin-3-O-gallate)	0.5	442.0910	441.0831
7.83	Procyanidin B2 gallate	3.6	882.1643	881.1602
7.87	2″-O-Galloylhyperin	1.9	616.1064	615.1003
9.54	Quercetin-3-O-β-D-glucuronide	0.9	478.0747	477.0679
9.83	6-Hydroxykaempferol-3-O-glucoside	0.5	464.0955	463.0884
9.91	1β,3β,6α-Trihydroxy-4α(15)-dihydrocostic acid methyl ester-1 -O-β-D-glucopyranoside	0.1	460.2309	459.2236
13.7	Kaempferol-3-O-β-D-glucopyranoside	0.4	448.1006	447.0935
17.8	Quercetin	-1.4	302.0427	301.0351
24.4	Rhamnetin	-1.8	316.0583	315.0505

https://doi.org/10.26656/fr.2017.8(S4).6

they can be promoted as a source of planting material source for breeding, conservation and commercial purposes. Further studies are also warranted to isolate and purify the bioactive constituents of *P. minor* and elucidate the underlying mechanisms associated with the beneficial properties. This information is critical in an effort to develop antioxidant supplementation and healthcare products in the future.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors wish to thank the Malaysian Agricultural Research and Development Institute (PIC-1001-507) for the financial support and facilities in conducting this research.

References

Abdullah, M.A., Mohd Ali, J., Abolmaesoomi, M., Abdul-Rahman, P.R. and Haji Hashim O. (2017). Anti-proliferative, in vitro antioxidant, and cellular antioxidant activities of the leaf extracts from *Polygonum minus* Huds: Effects of solvent polarity. *International Journal of Food Properties*, 20(Suppl. 1), 846-862. https://doi.org/10.1080/10942912.2017.1315591

Abubakar, M.A., Zulkifli, R.B., Hassan, W.N., Shariff, A.H., Malek, N.A., Zakaria, Z. and Ahmad, F. (2015). Antibacterial properties of *Persicaria minor* (Huds.) ethanolic and aqueous-ethanolic leaf extracts. *Journal of Applied Pharmaceutical Science*. 5(Suppl 2), 50-56. https://doi.org/10.7324/JAPS.2015.58.S8

Anand David, A.V., Arulmoli, R. and Parasuraman, S. (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. *Pharmacognosy Reviews,* 10(20), 84–89. https://doi.org/10.4103/0973-7847.194044

Barchan, A., Bakkali, M., Arakrak, A., Pagán, R. and Laglaoui, A. (2014). The effects of solvents polarity on the phenolic contents and antioxidant activity of three *Mentha* species extracts. *International Journal of Current Microbiology Applied Sciences,* 3(11), 399–412.

Benzie, I.F.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry,* 239(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Christapher, P.V., Xin, T.Y., Kiun, C.F., Leng, L.C., Fu, N.G., Yuan, G.L., Parasuraman, S. and Vikneswaran, M. (2015). Evaluation of analgesic, anti-inflammatory, antipyretic and antiulcer effect of aqueous and methanol extracts of leaves of *Polygonum minus* Huds. (Polygonaceae) in rodents. *Archives of Medicine and Health Sciences*, 3(1), 12-17. https://doi.org/10.4103/2321-4848.154919

Irondi, E.A., Oboh, G., Akindahunsi, A.A., Boligon, A.A. and Athayde, M.L. (2015). Phenolics composition and antidiabetic property of *Brachystegia eurycoma* seed flour in high-fat diet, low-dose streptozotocin-induced type 2 diabetes in rats. *Asian Pacific Journal of Tropical Disease, 5* (S1), S159–S165. https://doi.org/10.1016/S2222-1808(15)60880-5

Kala, H.K., Mehta, R., Tandey, R., Sen, K.K. and Mandal, V. (2016). Ten years of research on phenolics (2005–2015): A status report. *Pacific Science Review A: Natural Science and Engineering,* 18(1), 1–4. https://doi.org/10.1016/j.psra.2016.07.002

Kassim, N.K., Rahmani, M., Ismail, A., Sukari, M.A., Cheng, G.L.E., Mohd Nasir, N. and Awang, K. (2013). Antioxidant activity-guided separation of coumarins and lignan from *Melicope glabra* (Rutaceae). *Food Chemistry*, 139(1–4), 87–92. https://doi.org/10.1016/j.foodchem.2013.01.108

Krishnasamy, G. and Muthusamy, K. (2015). *In vitro* evaluation of antioxidant and antidiabetic activities of *Syzigium densiflorum* fruits. *Asian Pacific Journal of Tropical Disease,* 5(11), 912–917. https://doi.org/10.1016/S2222-1808(15)60956-2

Kua, Y.L., Gan, S., Morris, A. and Ng, H.K. (2016). Ethyl lactate as a potential green solvent to extract hydrophilic (polar) and lipophilic (non-polar) phytonutrients simultaneously from fruit and vegetable by-products. *Sustainable Chemistry and Pharmacy,* 4, 21–31. https://doi.org/10.1016/j.scp.2016.07.003

Lau, H., Shahar, S., Mohamad, M., Rajab, N.F., Yahya, H.M., Din, N.C. and Hamid, H.A (2020). The effects of six months *Persicaria minor* extract supplement among older adults with mild cognitive impairment: a double-blinded, randomized, and placebo-controlled trial. *BMC Complementary Medicine and Therapies*, 20(1), 315. https://doi.org/10.1186/s12906-020-03092-2

Lee, Y.L., Tsung, Y.M. and Mau, J.L. (2007). Antioxidant properties of various extracts from *Hypsizigus marmoreus*. *Food Chemistry*, 104(1), 1-9. https://doi.org/10.1016/j.foodchem.2006.10.063

León-Buitimea, A., Garza-Cárdenas, C.R., Garza-Cervantes, J.A., Lerma-Escalera, J.A. and Morones-

Ramírez, J.R. (2020). The demand for new antibiotics: Antimicrobial peptides, nanoparticles, and combinatorial therapies as future strategies in antibacterial agent design. *Frontiers in Microbiology*, 11, 1669. https://doi.org/10.3389/fmicb.2020.01669

Miklasińska-Majdanik, M., Kępa, M., Wojtyczka, R.D., Idzik, D. and Wąsik, T.J. (2018). Phenolic compounds diminish antibiotic resistance of *Staphylococcus aureus* clinical strains. *International Journal of Environmental Research and Public Health*, 15(10), 2321. https://doi.org/10.3390/ijerph15102321

Mirfat, A.H.S., Amin, I., Nur Kartinee, K., Muhajir, H. and Mohd. Shukri, M.A. (2020). Phenolic profiling and evaluation of in vitro antioxidant, α-glucosidase and α-amylase inhibitory activities of *Lepisanthes fruticosa* (Roxb.) Leenh. fruit extracts. *Food Chemistry*, 331, 127240. https://doi.org/10.1016/j.foodchem.2020.127240

Mohd Effendi, M.N., Mirfat, A.H.S., Ainon Dzahirah, Z., Nur Ilida, M., Muhammad Faidhi, T., Muhammad Faris, M.R. and Ahmad Syahman, M.D. (2022). Promising antibacterial activity from different accessions of kesum (*Persicaria minor* (Huds.) Opiz), presented at the Proceedings of MARDI Science and Technology Exhibition 18 - 19 October, p. 86. Serdang, Selangor, Malaysia: MARDI.

National Committee for Clinical Laboratory Standards (1999). Handbook of Methods. 2nd ed. UK: CAB International.

Passo Tsamo, V.C., Herent, M.F., Tomekpe, K., Emaga, T.H., Quetin-leclercq, J., Rogez, H., Larondelle, Y. and Andre, C. (2015). Phenolic profiling in the pulp and peel of nine plantain cultivars (*Musa* sp.). *Food Chemistry,* 167, 197–204. https://doi.org/10.1016/j.foodchem.2014.06.095

Vimala, S., Rohana, S., Rashih, A.A. and Juliza, M. (2011). Antioxidant evaluation in Malaysian medicinal plant: *Persicaria minor* (Huds.) leaf. *Science Journal of Medicine and Clinical Trials,*1, 9-16.

Yahya, H., Shahar, S., Ismail, S., Aziz, A., Normah, C.D. and Hakim, B.N. (2017). Mood, cognitive function and quality of life improvements in middle aged women following supplementation with *Polygonum minus* extract. *Sains Malaysiana,* 46, 245-254. https://doi.org/10.17576/jsm-2017-4602-09

