Determination of antibacterial activity of six accessions of *Persicaria minor* (Huds.) Opiz (kesum) extracts from different extraction solvents

[1]Mohd Effendi, M.N., [1]Ainon Dzahirah, Z., [1]Mirfat, A.H.S., [1]Muhammad Faidhi, T., [1]Muhammad Faris, M.R. and [1]Ahmad Syahman, M.D.

[1]Industrial Crops Research Centre, Malaysian Agriculture Research and Development Institute (MARDI) Headquarters, 43400 Serdang, Selangor, Malaysia.

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Abstract: *Persicaria minor* (Huds) Opiz or locally known as kesum is a popular aromatic herb used as food additive to enhance flavour. Several studies reported its biological activities such as antioxidant, antimicrobial, antivirus, anti-inflammatory, antiulcer and anticytotoxicity. However, there is lack of literature review on antibacterial activity of different accessions of *P. minor* extracted using different solvents. The present study was aimed to evaluate the antibacterial activity of 6 accessions of *Persicaria minor*: MKSM002, MKSM004, MKSM006, MKSM011, MKSM013, MKSM020, extracted using 70% methanol, dichloromethane, and ethyl acetate against five pathogens: *Bacillus cereus* ATCC 10876, *Cronobacter sakazakii* ATCC 29544, *Enterobacter aerugenes* ATCC 13048, *Listeria innocua* ATCC 33090 and *Staphylococcus aureus* ATCC 25923 using disc diffusion method. Extraction using 70% methanol produced the highest yield. All extracts exhibited promising antibacterial activity with various inhibition zones against all pathogen tested. Among three solvents and six accessions, 70% methanol extract of MKSM002, MKSM004 and MKSM006 showed the widest inhibition zone against *E. auregenes* and *S. aureus*. Methanol extract of MKSM004 also showed highest activity against *L. innocua* followed by MKSM011 methanol extract against *E. aerugenes* and *L. innocua*. Dichloromethane MKSM006 extract showed similar strength of the activity against *S. aureus*. While the other extracts showed a significantly lower inhibition zone with a diameter of the inhibition zone between 7.25 to 10.50 mm for all pathogens tested. The commercial antibiotic Penicillin used as positive control only inhibited *C. sakazakii*, *E. aerugenes* and *S. aureus*. For minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC), 70% methanol extract of *P. minor* accessions recorded the lowest concentration compared to dichloromethane and ethyl acetate extracts. This study suggests that *P. minor* accessions are promising antibacterial agent and 70% methanol is the most suitable solvent to extract compounds from *P. minor* for biological activity study.

Keywords: accession, antimicrobial, aromatic herb, pathogen, solvents

1. Introduction

Studies on the use of herbs as traditional medicine have been actively conducted. The World Health Organization (WHO) has recommended traditional medicine based on natural ingredients in medical policies (Mushore & Matuvhunye, 2013). This is because there is a demand for safer and natural drugs or preservatives. In addition, the increase in cases of microbial resistance to antibiotics is also making the world search for new sources of antimicrobial agent based on natural ingredients. Antibiotic-resistant bacteria are a serious problem in the medical field. This cause difficulty in treating diseases caused by microorganisms. Some bacteria have developed resistance to antibiotics that were once commonly used to treat them. For example, *Staphylococcus aureus* ('golden staph' or MRSA) and *Neisseria gonorrhoeae* are now almost always resistant to benzyl penicillin. Benzyl penicillin also has little effect on most microorganism found in human digestive gut (Better Health Channel, 2021). The U.S. Food and Drug Administration (FDA) has approved many plant herbs for medicinal and therapeutic purposes (Kraisintu, 2003). Plenty of studies have been conducted in evaluating antimicrobial property of plants. For example, study by Nascimento et al. (2000) reported about antibacterial activity of plant extracts from following plants; *Achillea millifolium* (yarrow), *Caryophyllus aromaticus* (clove), *Melissa offficinalis* (lemon-balm), *Ocimun basilucum* (basil), *Psidium guajava* (guava), *Punica granatum* (pomegranate), *Rosmarinus officinalis* (rosemary), *Salvia officinalis* (sage), *Syzygyum joabolanum* (jambolan) and *Thymus vulgaris* (thyme). Several studies also reported antimicrobial property of herbs such

as misai kucing / cat's whiskers (*Orthosiphon stamineus*), longevity spinach (*Gynura procumbens*), mas cotek (*Ficus deltoidea*) (Ashraf et al., 2020), black ginger (*Kaempferia parviflora*) (Sitthichai et al., 2022), *Centella asiatica* (Suzita and Jun Xian, 2021) and kesum (Persicaria minor) (Musa Ahmed et al., 2015).

Foodborne pathogens are the main cause of foodborne diseases or known as food poisoning that caused by consumption of food or water contains pathogenic microbes. Among the food pathogens commonly associated with food poisoning are opportunistic bacteria of *Proteus*, *Klebsiella*, *Enterobacter*, *Vibrio*, *Pseudomonas*, *Salmonella*, *Listeria monocytogenes*, *Escherichia coli* and toxin-producing bacteria like *Clostridium botulinum*, *Staphylococcus aureus* and *Bacillus cereus* (Pavel, 2020). The degree of infection depends on the virulence and pathogenic type of bacteria ranging from mild infections such as vomiting, nausea, rash, skin itching to life-threatening ones such as bacteraemia, endocarditis, necrotizing pneumonia, toxic shock syndrome and food poisoning (Lin and Peterson, 2010).

The accession of an herb refers to the locality of an herb obtained or collected. Herbs from different localities are found to have different compositions, constituents and characteristics of the plant besides different vernacular names to plants of the same species based on where they grow from (James et al., 2008). This differences in constituents of the plants affected by several factors such as variations in climate, altitude, environmental conditions, type of soil, temperature, humidity, shading, location which also affected secondary metabolites of a plant (Bourgaud et al., 2001). Study by Zainol et al. (2003) reported that accessions affect the composition of phenolic compounds in *Centella asiatica*. To this date, there is limited study on different accessions of *Persicaria minor* in Malaysia or Southeast Asia.

The selection of solvents for herbal plant extracts is very important in determining all the phenolic compounds extracted into the solvent in order to determine their biological activities. The types of solvents are polar, semi-polar and non-polar. These types of solvents determine the type of phenolic compounds to be extracted (Pinelo et al. 2004) and solute into the solvents (Saad et al. 2014).

Persicaria minor (Huds.) Opiz, or also has synonym name *Polygonum minus*, is an edible aromatic herb belongs to the Polygonacea family and native to Southeast Asia including Malaysia, Thailand, Vietnam, and Indonesia. In Malaysia and Indonesia, it is called *kesum* and popularly used as a flavouring in culinary as well as traditional medicine to treat dandruff, stomach indigestion, and fungal infections (Musa Ahmed et al. 2015, Vimala et al. 2011). It also has a potential as an antiulcer, antimicrobial, anticytotoxicity and anti-genotoxicity (Qader et al. 2011; Uyub et al. 2010; Wasman et al. 2010). Several studies have determined *P. minor* possess antibacterial property against pathogenic bacteria (Musa Ahmad et al. 2015, Nagi et al. 2018, Ridzuan et al. 2013) other than strong antioxidant (Norsyamimi et al. 2014; Vimala et al. 2011). However, there is lack of literature review on different accessions of *P. minor* and its antibacterial activity for each accession. In this study, antibacterial activity of 6 accessions of *P. minor* extracted using different extraction solvents were screened.

2. Materials and methods

Collection and preparation of plant material

P. minor accessions were collected from various localities around the state of Selangor, Malaysia randomly. Two accessions were obtained from commercial planting plots using bordered planting and stagnant containers, while the remaining accessions lived in water drainage in villages. Two types of growth habits identified in the accessions that have been collected are erect and decumbent types. The collection information and types of accessions collected are as in table 1. All cuttings of each accession were replanted in the herb nursery plot in MARDI Serdang, Selangor, Malaysia for further growth and sampling. Those six accessions of *P. minor* were identified as MKSM002, MKSM004, MKSM006, MKSM011, MKSM013, and MKSM0020. All accessions were harvested at optimal maturity which was around 16 weeks after planting. The whole plants excluded root were cleanse and cut into smaller pieces and dried at 50 °C in the oven for 3 days until moisture of the sample reached 10% to prevent the growth of fungal. The dried samples were then ground into powder for extraction.

Table 1. P. minor accessions information collected from various localities.

Accession	Locality	Latitude	Longitude	Growth habit	Habitat
MKSM002	Sabak Bernam	N 3°36'12.4"	E 101°03'43.6"	Decumbent	Water drainage
MKSM004	Kuala Selangor	N 3°27'08.6"	E 101°09'12.7"	Erect	Water drainage
MKSM006	Kuala Selangor	N 3°15'59.4"	E 101°20'51.1"	Decumbent	Commercial open field planting
MKSM011	Kuala Langat	N 2°53'20.7"	E 101°33'11.2"	Erect	Water drainage
MKSM013	Klang	N 3°04'29.6"	E 101°23'44.1"	Decumbent	Commercial stagnant container
MKSM020	Hulu Langat	N 3°05'30.9"	E 101°47'26.9"	Decumbent	Water drainage

Preparation of crude extract

An amount of 10g of each accession of *P. minor* powder was extracted in 100ml of different solvents; 70% (v/v) methanol, 99.9% (v/v) dichloromethane, and 99.9% (v/v) ethyl acetate in round bottom flask. The mixtures were shaken using orbital shaker at 200 rpm for 3 days in room temperature. The extracts were filtrated through Whatmann filter paper No. 1 and dried using rotary evaporator. Crude extracts were then weighted and diluted in dimethyl sulfoxide (DMSO) to achieve 100 mg/mL extract stock.

Screening of antibacterial activity

Antibacterial activity of all extracts was determined using disc diffusion technique according to method by Bauer et al. (1966), against five pathogen strains: *Bacillus cereus* ATCC 10876, *Cronobacter sakazakii* ATCC 29544, *Enterobacter aerugenes* ATCC 13048, *Listeria innocua* ATCC 33090 and *Staphylococcus aureus* ATCC 25923. All cultures were obtained from Biohazard Laboratory of Food Technology Research Centre, MARDI Serdang, Selangor, Malaysia. An aliquot of 100 µL from each strain culture stock was cultivated overnight in 9 mL sterile nutrient broth prior to use. The fresh grown strains then diluted with sterile saline solution to get inoculum concentration at 1 x 10^8 CFU/mL as compared to McFarland standard turbidity. An amount of 100 µL of the standardized bacterial inoculum was pipetted on top of the solidified Mueller Hinton agar (MHA) in the petri dish and streaked evenly using a sterile cotton swab. Sterile 6 mm diameter blank paper discs were placed over the surface of the MHA using sterile forceps. An aliquot of 20 µL of each extract solution was impregnated carefully on the paper disc and left to dry. The commercial antibiotic Penicillin disc (10 µg) (Oxoid) was used as a positive control. All these petri dishes were then incubated at 37 °C for 24 hours. Formation of clear zone around the paper disc was measured and recorded.

Determination of minimum inhibitory concentration (MIC)

The MIC of all extracts was carried out using microdilution method. The extracts at 100 mg/mL were subjected to two-folded serial dilution, to obtain a range of concentrations of 100 - 0.19 mg/mL. An aliquot of 100 µL of previously prepared standardized bacterial inoculum was added to 96-well microplate, before mixed with 100 µL of prepared diluted extract. The microplate was then incubated at 37 °C for 24 hours. The wells were then observed for the presence or absence of visible turbidity. The lowest concentration that showed no turbidity (no growth of bacterial) was reported as MIC (Musa Ahmed et al., 2015).

Determination of minimum bactericidal concentration (MBC)

An amount of 100 µL from the mixture in microplate wells from MIC test was pipetted onto solidified Mueller Hinton agar and spread evenly using sterile cotton swab. The inoculated plates were then incubated at 37 °C for 24 hours. The lowest concentration that did not show bacterial growth was considered as MBC (Musa Ahmed et al., 2015).

Statistical analysis

Data from three replicates of each sample were used for statistical analysis. The data analysis was done using Analysis of Variance (ANOVA) IBM SPSS software version 26. Mean comparison between samples and treatments was obtained using Duncan's multiple range test at $p < 0.05$ level.

3. Results and discussion

Antibacterial activity of six accessions of *P. minor* or kesum extracted using 70% (v/v) methanol, 99.9% (v/v) dichloromethane and 99.9% (v/v) ethyl acetate, against five bacterial strains is shown in Table 1. The range of inhibition zone diameter for all extracts was between 7.25 – 15.75 mm. The widest inhibition zones were exhibited by 70% methanol extract of MKSM002 (14.12mm), MKSM004 (14.25mm) and MKSM006 (15.75mm) accessions against *E. aerugenes*, significantly ($p<0.05$) higher than the rest of the extracts. Followed by this, the same extracts against *S. aureus* (11, 11.75 and 10.75mm) showed second highest of inhibition. For other solvents, only dichloromethane extract of MKSM006 showed high inhibition, against *S. aureus* (11.25mm). The rest of the extracts exhibited lower inhibition diameter ranged from 7.25 – 10.75 mm ($p>0.05$). The inhibition zone indicates the susceptibility of the microbes to the extract. Inhibition zones with a size exceeding 7 mm in diameter indicate microbes susceptible to the extract, meaning the extract has antimicrobial activity (Nascimento et al. 2000). The same range of inhibition zone diameter was observed in methanol and dicholoromethane *P. minor* extracts against *S. aureus* and *S. epidermidis* in study done by Ridzuan et al. (2013). In the same study also revealed *P. minor* methanol extract showed higher antibacterial activity compared to dichloromethane extract in all extract concentrations used. Bioactive compounds such as phenolics, flavonoids, alkaloids, aldehydes, and terpenoids contribute to antimicrobial activity of kesum (Imelda, 2014). Ridzuan et al. (2013) discovered 28 major compounds from kesum leaves extract with the highest compounds were dodecanal, decanal, α-citral, drimenol, Z-citral, caryophyllene, euporone, and 2,4-heptadiene,2,6-dimethyl while Sasongko et al. (2011) found 28 volatile compounds in extracts of fresh and dried kesum where the major compounds were eupatoriochromene, dodecanal, alpha-caryophyllene, beta-caryophyllene, and decanal.

Table 1: Inhibition zone of 70% (v/v) methanol, 99.9% (v/v) dichloromethane and 99.9% (v/v) ethyl acetate extracts of 6 accessions of *P. minor.*

Solvent	Accession	Diameter of inhibition zone (mm)				
		B. cereus	*C. sakazakii*	*E. aerugenes*	*L. innocua*	*S. aureus*
70% methanol	MKSM002	8.25±1.50ghi	7.50±0.57hi	14.12±0.25b	9.75±0.50cdefg	11.00±0.81cd
	MKSM004	9.75±0.50cdefg	7.25±0.50i	14.25±2.98b	10.62±3.19cde	11.75±0.50c
	MKSM006	8.25±0.50ghi	8.25±0.50ghi	15.75±3.77b	9.00±0.81defghi	10.75±0.95cde
	MKSM011	9.50±0.57defgh	9.25±0.50defghi	10.50±1.00cdef	10.50±0.57cdef	9.75±0.50cdefg
	MKSM013	8.75±0.50efghi	9.00±0.81defghi	9.00±0.00defghi	8.75±0.50efghi	9.75±0.50cdefg
	MKSM020	8.50±0.57fghi	9.50±1.29defgh	8.25±0.50ghi	8.00±0.81ghi	8.50±0.57fghi
Dichloro methane	MKSM002	8.75±0.95efghi	8.00±0.00ghi	9.00±0.81defghi	8.25±0.50ghi	9.00±0.81defghi
	MKSM004	8.25±0.50ghi	8.50±0.57fghi	9.00±0.81defghi	7.50±0.57hi	8.50±0.57fghi
	MKSM006	8.00±0.81ghi	9.00±0.00defghi	9.75±0.50cdefg	9.00±0.81defghi	11.25±0.50c
	MKSM011	8.75±0.95efghi	10.25±0.50cdef	9.75±0.50cdefg	9.00±0.00defghi	9.75±0.50cdefg
	MKSM013	8.75±0.95efghi	9.75±0.95cdefg	9.00±0.81defghi	8.75±0.95efghi	10.50±0.57cdef
	MKSM020	7.75±1.50ghi	8.00±0.81ghi	8.50±0.57fghi	8.25±0.95ghi	9.25±0.50defghi
Ethyl acetate	MKSM002	8.75±0.95efghi	7.75±0.95ghi	9.25±1.50defghi	8.00±1.15ghi	8.75±0.50efghi
	MKSM004	8.87±0.62efgh	8.50±0.57fghi	9.00±1.41defghi	8.00±0.81ghi	8.75±0.50efghi
	MKSM006	8.51±0.57fghi	8.00±0.00ghi	9.50±1.29defgh	7.75±0.50ghi	9.50±0.57defgh
	MKSM011	8.50±0.57fghi	8.25±0.50ghi	8.75±0.50efghi	7.75±0.50ghi	8.25±0.50ghi
	MKSM013	7.50±0.57hi	8.75±1.50efghi	8.75±0.50efghi	8.00±0.00ghi	8.75±0.50efghi
	MKSM020	9.00±1.15defghi	7.50±0.57hi	8.25±0.50ghi	8.50±0.57fghi	9.25±0.50defghi
Penicillin		0j	11±0.00cd	10±0.00cdef	0j	40±0.57a

Values are presented in the mean value of four replicates (mean±standard deviation). Different letters in the same row and column indicate difference significant ($p<0.05$

In antimicrobial study, minimum inhibitory concentration (MIC) refers to the lowest concentration of extract that will inhibit microbial growth, while minimum bactericidal concentration (MBC) indicates the lowest concentration of the extract that can kill tested microbes (Mehta et al. 2013). The MIC and MBC values of 70% (v/v) methanol, 99.9% (v/v) dichloromethane and 99.9% (v/v) ethyl acetate six *P. minor* accessions are shown in Table 2 and 3, respectively. It was observed that 70% methanol extracts of all accessions exhibited the lowest MIC against three bacterial strains: *S. aureus* (0.78 – 6.25 mg/mL), *E. aerugenes* (0.19 – 1.56 mg/mL) and *B. cereus* (6.25 mg/mL). Variation of MIC and MBC values were observed among accessions in 70% (v/v) methanol extract against *S. aureus* and *E. aerugenes*, compared to against *B. cereus*. For 99.9% (v/v) dichloromethane and 99.9% (v/v) ethyl acetate extracts, their lowest concentration showed against *E. aerugenes* (0.78 – 1.56 mg/mL) while *S. aureus* and *B. cereus* showed higher MIC (25 and 50 mg/mL respectively). Dichlomethane and ethyl acetate extracts showed variation of MIC values among accessions against *E. aerugenes*, but the values are similar against *S. aureus* and *B. cereus*. Meanwhile for MBC, 70% (v/v) methanol extracts of all accessions exhibited the lowest MBC against *S. aureus* (1.56 – 12.50 mg/mL) and *E. aerugenes* (0.19 – 1.56 mg/mL) compared to other two solvents where its lowest MBC is against *E. aerugenes* (0.78 – 1.56 mg/mL) followed by dichloromethane and ethyl acetate against *S. aureus* (25 mg/mL) and *B. cereus* (50 mg/mL). These findings are very similar to the results in study conducted by Ridzuan et al. (2013) where MIC value in kesum methanol extract was lower (3.125 mg/mL) compared to dichloromethane extract (12.5 mg/mL). Meanwhile study done by Nagi et al. (2018) showed higher MIC and MBC values of kesum extracts which were 62.50 – 250 mg/mL. The diversity of the values in inhibition zones, MIC and MBC implies the strength and differences of phytochemical compounds in an herbaceous plant against a particular bacterial strain (Saad et al. 2014). The different physiology of the bacteria species could result the ability of the herbal extract to interfering the growth, thus contributing to the variation in antibacterial activity. The solvent type plays an important role in extraction process, where phenolic solubility depends on solvent polarity (Naczk & Shahidi, 2006). There are differences in the way of active compounds react when polar, less polar or non-polar solvents are used (Pinelo et al. 2004). High-polar phenolic compounds will be soluble in polar solvents, while less polar compounds will be soluble in non-polar solvents. Several active compounds of different polarity may be present in varying amounts in the extract. Methanol and ethanol at 70% (v/v) concentration were observed extracting more phenolics and flavonoids (Norsyamimi et al. 2014). Plant secondary metabolites and phenolic compounds can be affected by the accessions of the plant and where the plant grow from (James et al., 2008). Environmental conditions where the plants grow like type of soil, temperature, humidity, shading, altitude and location have been reported to have effect on secondary metabolites of a plant (Bourgaud et al., 2001). This may explain why *P. minor* accessions exhibited different and diverse antibacterial activities. May this present study be reference and guidance in selection of best *P. minor* accession in promoting *P. minor* cultivation and herbal products development.

Table 2: Minimum inhibition concentration (MIC) values of 70% (v/v) methanol, 99.9% (v/v) dichloromethane and 99.9% (v/v) ethyl acetate extract of six *P. minor* accessions

Solvent	Accession	MIC value (mg/mL)		
		S. aureus	*E. aerugenes*	*B. cereus*
70% methanol	MKSM002	0.78	0.19	6.25
	MKSM004	6.25	1.56	6.25
	MKSM006	0.78	0.39	6.25
	MKSM011	3.12	0.19	6.25
	MKSM013	6.25	0.39	6.25
	MKSM020	6.25	0.78	6.25
Ethyl acetate	MKSM002	6.25	0.78	50
	MKSM004	12.50	0.78	50
	MKSM006	12.50	1.56	50
	MKSM011	12.50	1.56	50
	MKSM013	12.50	1.56	50
	MKSM020	12.50	1.56	50
Dichloromethane	MKSM002	12.50	0.78	50
	MKSM004	12.50	1.56	50
	MKSM006	12.50	1.56	50
	MKSM011	12.50	0.78	50
	MKSM013	12.50	0.78	50

	MKSM020	12.50	1.56	50

Table 3: Minimum bactericidal concentration (MBC) values of 70% methanol, dichloromethane and ethyl acetate extract of six *P. minor* accessions

Solvent	Accession	MBC value (mg/mL)		
		S. aureus	*E. aerugenes*	*B. cereus*
70% methanol	MKSM002	1.56	0.19	50
	MKSM004	12.50	1.56	50
	MKSM006	1.56	0.39	50
	MKSM011	6.25	0.19	50
	MKSM013	6.25	0.39	50
	MKSM020	12.50	0.78	50
Ethyl acetate	MKSM002	25	0.78	50
	MKSM004	25	0.78	50
	MKSM006	25	1.56	50
	MKSM011	25	1.56	50
	MKSM013	25	1.56	50
	MKSM020	25	1.56	50
Dichloromethane	MKSM002	25	0.78	50
	MKSM004	25	1.56	50
	MKSM006	25	1.56	50
	MKSM011	25	0.78	50
	MKSM013	25	0.78	50
	MKSM020	25	1.56	50

Conclusion

All six accessions of *P. minor* have exhibited good and potentially antibacterial activities against five pathogenic bacteria tested. Accession MKSM002, MKSM004 and MKSM006 showed the strongest activity. For extraction solvent, based on antibacterial screening, MIC and MBC values, 70% methanol was shown to be the best solvent to extract the phenolic compounds from *P. minor* plant for testing its biological activity. With this screening discovery, *P. minor* could be utilised further to develop new antimicrobial agent.

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