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Polyphenol extraction from mangosteen (*Garcinia mangostana* Linn) pericarp by bio-based solvents

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Abstract

Mangosteen pericarp (MP), an abundant agro-food waste in Thailand, can be used to produce polyphenols. In the present work, the effects of bio-based solvents (virgin coconut oil, VCO; propylene glycol, PG; glycerol, and water) in comparison with organic solvents (ethanol and hexane) on extraction and *in vitro* bioactivities of phenolic compounds from MP were evaluated. Solvents strongly influenced the total phenolic content (TPC), total flavonoid content (TFC), phenolic profiles, antioxidant activity, antibacterial activity, and antidiabetic activity of the extracts. Overall, α-mangostin and γ-mangostin were the major phenolic constituents found in all extracts. PG was the optimal solvent yielding the highest TPC and TFC with a broad spectrum of phenolic profiles and rendering the extract with the highest antioxidant activity, antibacterial activity, and antidiabetic activity. However, the antibacterial activity of the extract was enhanced when VCO was used. Thus, bio-based solvents, particularly PG, were suitable for the recovery of active phenolic compounds form MP.

Introduction

Polyphenols are the most abundant phytochemicals with several important bioactivities and their demand have significantly increased in the global market. Typically, phenolic compounds can be extracted from the fruits, leaves, roots, and inedible plant materials e.g. peels. Peel fractions from some fruits showed higher antioxidant activity than the pulp fractions. Arazo *et al.* (2011) reported that mangosteen pericarp (MP) exhibited higher antioxidant activity than its pulp. Generally, MP accounts for approximately 60% of the waste from mangosteen-based processing from wine, jam, puree, and fresh cut production. A variety of phenolic compounds e.g. condensed tannins, anthocyanins, xanthones, and other phenolic compounds have been identified from MP. The xanthone group, enriched in MP, particularly α- and γ-mangostin, exhibited antioxidant activity, antibacterial activity (Suksamrarn *et al.,* 2002; Pothitirat *et al.,* 2009), antidiabetic activity (Adnyana *et al.,* 2016) and other bioactivities such as antitumor, anti-inflammatory, anti-allergenic, neuroprotective activity, anti-ulcer, antimalarial, anti-acne, cytoprotective activity, anticancer, and histamine receptor blockers (Zarena and Udaya Sankar, 2009a; Chen *et al.,* 2018). Due to its pharmacological activities and its large quantity, the MP extract is popularly applied as a functional ingredient for herbal cosmetics, pharmaceuticals, nutraceuticals, and functional food products.

Polyphenol separation is commonly assisted by organic solvent extraction. Types of extraction solvent and solvent concentration were reported as an important factor influencing the rate of extraction and quality of the resulting extract (Zarena and Udaya Sankar, 2009b). Several solvents have been recommended for extracting phenolic compounds from MP, such as ethyl acetate, acetone, dichloromethane, hexane, methanol, ethanol, and benzene (Zarena and Udaya Sankar, 2011). Recently, there has been an increase in the application of bio-based media for the recovery of phenolic compounds from plant origins because organic solvents are now strictly regulated worldwide. The utilisation of bio-based solvents for extracting the phenolic compounds from MP is one of the strategies to improve its beneficial property, and it can be used directly in food and personal care products without removal of solvent.

Vegetable oils have been reported as alternative solvents for green oleo-extraction, purification, and formulation of food and natural products (Yara-Varón *et al.,* 2017). However, there is limited information regarding the use of vegetable oils for the recovery of MP phenolic compounds (Krishna *et al.,*

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2010). Virgin coconut oil (VCO), as a bio-based solvent, is a possible viable alternative to other commercial edible oils. VCO has superior chemical properties as a solvent in comparison with other vegetable oils (Krishna *et al.,* 2010). VCO, and medium chain triglycerides (MCT) from VCO, are widely used in the flavour industries because of their higher polarity as compared to conventional fats and oils (Krishna *et al.,* 2010). These optimal polarity properties make VCO and MCT superior carriers for flavour, vitamins, and colours when compared with other natural oils. VCO has a lower molecular weight than other natural oils, and this results in a lower viscosity, even at low temperatures as compared to other natural oils (Krishna *et al.,* 2010). Pujirahayu *et al.* (2015) showed that propolis, containing a variety of chemicals such as polyphenols, terpenoids, and amino acids, can be extracted by VCO and olive oil. These extracts had higher antibacterial activity than the ethanol extract (Pujirahayu *et al.,* 2015). VCO is exceptionally stable, with little oxidation due to only small amounts of unsaturated fatty acids. Consequently, VCO has excellent keeping qualities and this helps increase the shelf-life of the finished products (Krishna *et al.,* 2010). VCO itself has a high nutritional value and bioactivities with little or no adverse effects on human health as compared to other oils. Furthermore, VCO is also rich in lauric acid and monolourin, which inhibit various pathogenic bacteria such as *Listeria monocytogenes*, enterotoxigenic *Escherichia coli* and *Staphylococcus aureus* (Loung *et al.,* 2014). It also contains some phenolic compounds such as protocatechuic acid, vanillic acid, syringic acid, *p*-coumaric acid, caffeic acid, and ferulic acid (Srivastava *et al.,* 2016). VCO also has superior antioxidant potential as compared to the other oils (Marina *et al.,* 2009). Therefore, the utilisation of VCO as a bio-solvent for extracting bioactive compounds from natural resources is one of the strategies to improve beneficial properties of the compounds.

Generally, VCO, propylene glycol (PG) and glycerol have been used for the preparation of pharmaceutical and cosmetic products. However, there is very little research on the phenolic profile and *in vitro* bioactivities of MP as influenced by bio-based solvent extraction with varying polarity. Therefore, the present work aimed to explore the potential applications of bio-based solvents, including VCO, PG, glycerol, and water, for the recovery of phenolic compounds from MP in comparison with the conventional organic solvents, including ethanol and hexane. The recovery of phenolic compounds, phenolic profiles, and bioactivities of resulting MP extracts were then investigated.

Materials and methods

Preparation of VCO

VCO was prepared by fermentation. Briefly, coconut milk from mature coconut (*Cocos nucifera* Linn.) from the tall tree cultivar was stored at -18°C for 2 h. Thereafter, the upper cream layer was removed. To obtain VCO, coconut cream was incubated in a glass bowl at 35°C for 24 h. The oil phase was collected and then dried by using a rotary evaporator (Buchi, Rotavapor R-124) at 50-60°C under vacuum (100 mbar) for 15 min. The extracted VCO had a moisture content of 0.17 ± 0.01% (AOAC, 2000), iodine value of 6.10 ± 0.05 (AOAC, 2000), peroxide value of 0.64 ± 0.01 mEq/kg (AOAC, 2000), saponification number of 259 ± 1.0 mg KOH/g (AOAC, 2000), free fatty acids of 0.59 ± 0.01% (AOAC, 2000) and a *p*-anisidine value of 0.07 ± 0.04 (AOCS, 1999).

Preparation of the MP extract

The pericarp of fresh mature mangosteen (*Garcinia mangostana* Linn.) harvested from an agroforestry farm in Nakhon Si Thammarat, Thailand, was dried at 50°C using a tray dryer and ground using a Cyclotec™ 1093 Sample Mill (Foss Tecator Co., Hillerod, Denmark). After passing through a 100 mm mesh, the fine powder (10 g) was extracted with different bio-based media including VCO, PG, water, and glycerol. The bio-based extracts were compared with hexane and ethanol extracts prepared under continuous magnetic stirring (300 rpm) with dried powder per solvent ratio of 1:10 (w/v). Extractions were carried out in the Lib-300M incubator (Labtech, Korea) at 40°C for 24 h. Thereafter, the mixtures were centrifuged at 5,000 rpm for 10 min at ambient temperature (25 - 28°C) and the supernatants were collected. After flushing with nitrogen gas, the final extracts were frozen (-20°C) in air-tight amber bottles. The dielectric constant of hexane, VCO, ethanol, PG, glycerol, and water was reported as 1.9 (Wohlfarth, 2008), 2.8 (Mohamad *et al.,* 2014), 24.3 (Wohlfarth, 2008), 30.2 (Sengwa *et al.,* 2001), 39.2 (Akerlof, 1932) and 78.5 (Wohlfarth, 2008), respectively.

Separation of polyphenol fractions from MP extract

The MP phenolic compounds were separated from MP extract according to Nevin and Rajamohan (2006). A sample of MP extract was dissolved in 5 mL hexane and mixed with the same volume of methanol/water (60:40, v/v) and vortexed for 2 min.

Phase separation was performed by centrifugation at 3,500 rpm for 10 min and the hexane phase was re-extracted using the same method. The pooled fractions were analysed for total phenolic content (TPC), total flavonoid content (TFC), phenolic profile, antioxidant, and antidiabetic activity.

Determination of total phenolic content

The method of Gutfinger (1981) was used to estimate the TPC. Briefly, 100 µL of methanolic MP extract were added to 2.0 mL Folin-Ciocalteu reagent (previously diluted 10-fold with deionised water) and mixed well. Following incubation for 5 min, a 15% sodium carbonate solution (1.0 mL) was added. The solution was kept in the dark for 60 min. The absorbance was read at 765 nm using a Libra S22 UV-Visible spectrophotometer (Biochrom, Cambridge, England). TPC was expressed as milligrams of gallic acid equivalents (GAE) per 100 g of MP.

Determination of total flavonoid content

The TFC was determined following the method described by Meda *et al.* (2005), with slight modifications. Briefly, 5 mL of a 2% methanolic $AlCl_3$ solution was mixed with 5 mL of a methanolic MP extract. Following 10 min incubation, absorbance was read at 415 nm. A blank sample without $AlCl_3$ was used. The TFC was expressed as milligrams of rutin equivalents (RE) per 100 g of MP.

Determination of phenolic profiles

A reverse phase high performance liquid chromatography (HPLC) system (Prominence-I LC-2030C 3D Plus, Shimadzu, Kyoto, Japan) coupled with a Shim-pack GIST C_{18} column (4.6 × 150 mm, 5 µm size) and photodiode array detector were used to identify the phenolic constituents present in the MP extracts following the method of Owen *et al.* (2000).

Determination of reducing power

A modified Prussian blue assay was used to determine the reducing power of the extracts (Canabady-Rochelle *et al.,* 2015). Different concentrations of MP extracts were diluted 10-fold with 0.2 M phosphate buffer, pH 6.6. Seventy µL of sample solution was mixed with 35 µL of potassium ferricyanide (1%, w/v) and incubated at 50°C for 20 min. Thereafter, 135 µL of distilled water, 33 µL of trichloroacetic acid (10%, w/v), and 27 µL of ferric chloride (0.1% w/v) were added and incubated for 10 min at ambient temperature. Absorbance was read at 700 nm against a blank (without MP extract). Standards were prepared using ascorbic acid (AA) (0 - 100 µM). The reducing power was presented as milligrams of AA equivalents (AAE) per 100 g MP.

Determination of scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (DPPH•)

The method of Shimada *et al.* (1992) was used to evaluate the DPPH• scavenging activity. Briefly, 1 mL of MP extract (0.5 - 5.0 mg/mL) was mixed with 1 mL of 0.2 mM methanolic DPPH• solution. Following incubation in the dark at room temperature for 30 min, the absorbance was measured at 517 nm against a blank. DPPH• scavenging activity was calculated as:

$$\text{DPPH}^{\bullet}\text{ scavenging activity (\%)} = [(Abs_{Control} - Abs_{Sample})/Abs_{Control}] \times 100 \quad \text{(Eq. 1)}$$

where, $Abs_{Control}$ = absorbance of the control (without MP extract), and Abs_{Sample} = absorbance of the extract.

The half-maximal effective concentration (EC50; mg/mL) value was reported, and α-tocopherol was used as a comparison.

Determination of hydrogen peroxide (H2O2) scavenging activity

The method of Mukhopadhyay *et al.* (2016) was used to measure H_2O_2 scavenging activity. To a series of 96 well plates, 20 µL of ferrous ammonium sulphate (1 mM) was added to each well. Thereafter, 120 µL of different concentrations of MP extracts were serially diluted with deionised water. Fifty µL of 25 mM H_2O_2 was added and thoroughly mixed using a VM-300 vortex mixer (Gemmy Industrial Corp., Taipei, Taiwan). Following incubation in the dark for 5 min at room temperature, 120 µL of 1 mM 1,10-phenanthroline was added and mixed well. Absorbance was read at 510 nm using a BMG Labtech-SpectrostarNano plate reader (NC, USA) after incubating at room temperature for 10 min. The control solution was made with the same procedure, but distilled water was used instead of sample. The blank solution was also prepared; following the same protocol as control, but distilled water was added instead of H_2O_2. A reagent blank containing only 1,10-phenanthroline was prepared and the absorbance of the blank was subtracted from all extracts and standards. H_2O_2 scavenging activity of AA was used as a comparison. H_2O_2 scavenging activity was calculated using Eq. 2:

H_2O_2 scavenging activity (%) = [($Abs_{(Blank-Control)}$ – $Abs_{(Blank-Sample)}$)/$Abs_{(Blank-Control)}$] × 100 (Eq. 2)

The EC_{50} (mg/mL) value was also reported.

Determination of antibacterial activity

The minimal inhibitory concentration (MIC) of the individual MP extracts against the foodborne pathogen, *E. coli* and the major acne bacterium, *S. aureus* were determined by the agar dilution method (Topuz *et al.,* 2016), with some modifications. Sterile test tubes (20 mL) containing unsolidified tryptic soy agar with 0.6% yeast extract (TSAYE) and Mueller-Hinton agar (MHA) were prepared. MP extracts were diluted with dimethyl sulfoxide (DMSO) to obtain the desired phenolic concentrations (25 to 0.39 mg/mL). The corresponding diluted MP extract (500 µL) was pipetted into media test tubes. Thereafter, 100 µL suspensions (log 4.0 CFU/mL) of test bacteria were added to each tube. Prior to solidifications in plates at room temperature, the tubes containing test bacteria, MP extract and medium agar were mixed for 10 s. TSAYE and MHA with ampicillin (ranging from 125 to 1.95 µg/mL) and DMSO were used as positive and negative controls, respectively. Finally, plates were incubated at 37°C for 24 h and MIC values were reported.

In vitro α-amylase inhibitory assay

An *in vitro* α-amylase inhibitory assay was performed according to Ademiluyi and Oboh (2013). Briefly, the reaction mixture containing 500 µL of sodium phosphate buffer (100 mM, pH 6.9 plus 6.7 mM sodium chloride), 100 µL of porcine pancreatic α-amylase (1.0 unit/mL) (Sigma-Aldrich, St. Louis, USA) in 100 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride and 200 µL of MP extract (250 – 1,000 µg/mL) was pre-incubated in a 7.0 mL screw cap tube at 37°C for 20 min. Thereafter, a 200 µL 1% potato starch solution in sodium phosphate buffer, pH 6.9 was added as a substrate and incubated at 37°C for 30 min. The 3,5-dinitrosalicylic acid (DNS) colour reagent (1000 µL) was added to stop the reaction. DNS colour reagent solution contains 1.0 g of DNS, 29.9 g sodium potassium tartrate, 1.6 g sodium hydroxide and 100 mL deionised water. The tubes were subjected to a boiling water bath for 10 min. After cooling and adding 5 mL of distilled water, the absorbance was measured at 540 nm. Controls were prepared in an identical fashion without MP extract. Pure acarbose (Sigma-Aldrich, St. Louis, USA) at concentrations ranging from 2 - 10 µg/mL were used as positive controls. The inhibition of α-amylase was calculated using Eq. 3:

Alpha-amylase inhibitory activity (%) = [($\Delta Abs_{Control}$ – ΔAbs_{Sample})/$\Delta Abs_{Control}$] × 100 (Eq. 3)

where: $\Delta Abs_{Control}$ = Abs_{Test} (100% enzyme activity or only solvent with enzyme) - Abs_{Blank} (0% enzyme activity or solvent without enzyme); ΔAbs_{Sample} = Abs_{Test} (with enzyme) - Abs_{Blank} (A test sample without enzyme).

In vitro α-glucosidase inhibitory assay

An *in vitro* α-glucosidase inhibitory assay was performed according to Jayasri *et al.* (2009). Briefly, 50 µL of MP extract at various concentrations (250 - 1000 µg/mL) and 100 µL of 0.1 M phosphate buffer (pH-6.9) containing 0.25 unit/mL *Saccharomyces cerevisiae* α-glucosidase (Sigma-Aldrich, St. Louis, USA) were incubated in 96 well micro-plates at 25°C for 10 min. Thereafter, 50 µL of 5 mM *p*-nitrophenyl α-D-glucopyranoside (Sigma-Aldrich, St. Louis, USA) in 0.1 M phosphate buffer (pH-6.9) was added to each well at 5 s intervals. Following incubation at 25°C for 5 min, absorbance was read at 405 nm using a BMG Labtech-SpectrostarNano plate reader. The negative control was tested without MP extract. Pure acarbose at concentrations of 100 - 900 µg/mL, was used as a positive control. The inhibition of α-glucosidase was calculated using Eq. 4:

Alpha-glucosidase inhibitory activity (%) = [($Abs_{Control}$ – $Abs_{Samples}$)/$Abs_{Control}$] × 100 (Eq. 4)

Statistical analysis

Data were presented as means ± standard deviation from triplicate determinations. Statistical comparisons were performed using One Way Analysis of Variance (ANOVA) and by Duncan's new multiple range test (DMRT) using SPSS software. $p < 0.05$ indicated statistically significant difference.

Results and discussion

TPC and TFC

TPC and TFC of MP extracts extracted by different extracting solvents varying in dielectric constants are shown in Figure 1a and 1b, respectively. PG rendered the extract with the highest TPC (476 mg GAE/100 g), followed by glycerol, ethanol, water, VCO, and hexane ($p < 0.05$). For total

flavonoid recovery, the highest content was found in the ethanol extract (99 mg RE/100 g), followed by PG, VCO, glycerol/water, and hexane. The difference in phenolic and flavonoid recoveries among solvents might be due to the different polarity of phenolic and flavonoid constituents in MP. Using the edible bio-based solvent, VCO, the extract provided a TPC of 271 mg GAE/100 g and TFC of 49.3 mg RE/100 g. The TPC and TFC of the VCO extract were approximately 85 and 493 times higher than the TPC and TFC measured in the original VCO, respectively. The TPC and TFC of the original VCO was 3.2 mg GAE/100 g and 0.1 mg RE/100 g, respectively. Pothitirat *et al.* (2009) reported a TPC and TFC of an ethanolic MP extract of 28.88 ± 0.73 g GAE/100 g and 4.08 ± 0.07 g quercetin equivalents/100 g, respectively.

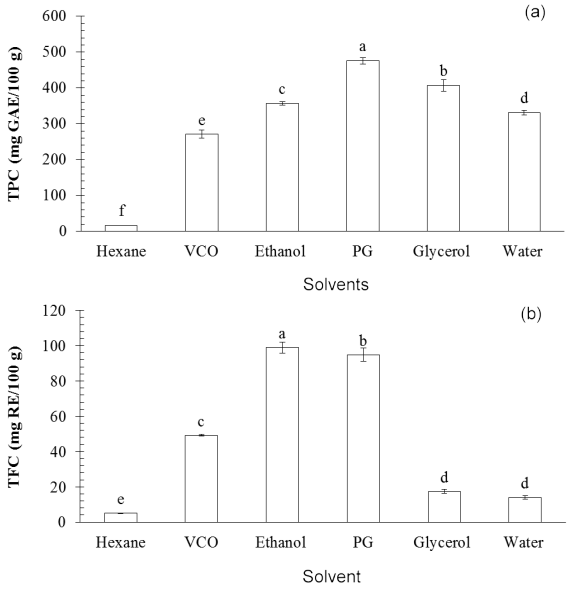

Figure 1. Total phenolic content (a) and total flavonoid content (b) of MP extracts as affected by different solvents. The bars represent standard deviation (SD) from triplicate determinations (n = 3). Different letters on the bars indicate statistically significant differences ($p < 0.05$). Total phenolic content and total flavonoid content of VCO were 3.20 ± 0.40 mg GAE/100 g and 0.10 ± 0.01 mg RE/100 g, respectively.

Phenolic profiles

Phenolics in plant extracts are often associated with other molecules such as proteins, polysaccharides, terpenes, chlorophyll, and inorganic compounds (Koffi *et al.,* 2010). Thus, it requires suitable solvents for the extraction of phenolic compounds. It is therefore not surprising that phenolic profiles of MP extracts varied based on the solvents used (Table 1). With a non-polar solvent, hexane (dielectric constant = 1.9), only α-mangostin and γ-mangostin were extracted. With VCO (dielectric constant = 2.8), xanthone was recovered together with a higher content of α-mangostin (10.4-fold) and γ-mangostin (5.5-fold), when compared to that extracted using hexane. With ethanol (dielectric constant = 24.3), a similar amount of xanthone was obtained as found in extracts using VCO, but α-mangostin and γ-mangostin contents were 27.3-fold and 9-fold higher than in the hexane extract. Vanillic acid was also found in the ethanol extract. Among solvents tested, ethanol provided the highest yield of α- and γ-mangostins ($p < 0.05$), followed by PG, VCO, hexane, water, and glycerol. The results indicated that ethanol was clearly more powerful for the quantitative extraction of α- and γ-mangostin from MP than PG, VCO, hexane, glycerol, and water. Ethanol is commonly used for extraction of α-mangostin from MP and enables a high recovery of α-mangostin in the extractant (Pothitirat et al., 2009). When PG (dielectric constant = 30.2) was used, the extract had a broad spectrum recovery of phenolic profiles including, α-mangostin, γ-mangostin, rutin, vanillic acid, (-) epicatechin, and trans-ferullic acid. The α- and γ-mangostin contents of the PG extract were less than that in the ethanol extract, but greater than that found in the other extracts.

The content of rutin was much higher in PG than in other extracts. PG is a chemical penetration enhancer in which the molecular structure generally contains both hydrophilic and lipophilic regions. An amphiphilic structure can enhance solubility and potentially separate out all polar and non-polar compounds from the plant membrane (Smith and Maibach, 1995). With glycerol (dielectric constant = 39.2) and water (dielectric constant = 78.5), the same phenolic profiles as those identified in PG extracts were apparent, but the quantities were significantly different. Quantities of vanillic acid, (-) epicatechin, and trans-ferullic acid were similar, but rutin, α-mangostin, and γ-mangostin were significantly lower when compared to that detected in the PG extract.

The representative HPLC chromatograms of the phenolic compounds in MP extracts obtained by VCO, ethanol, and PG are presented in Figure 2 (a-c). The HPLC fingerprints of the standard phenolic compounds are shown in Figure 2(d).

Antioxidant activity

The antioxidant activities of MP extracts as indicated by reducing power, DPPH$^{\bullet}$ scavenging activity, and H_2O_2 scavenging activity are summarised in Table 2. The highest reducing potential was found in the PG extract (209.7 ± 5.3 mg ascorbic equivalent/100 g), followed by extractions performed by glycerol, water, ethanol, VCO, and hexane

Table 1. The phenolic profiles of MP extracts as affected by different solvents.

Phenolic profile	Concentration (mg/100 g)					
	Hexane	VCO	Ethanol	PG	Glycerol	Water
(+) Catechin	ND[#]	ND	ND	ND	ND	ND
(-) Epicatechin	ND	ND	ND	4.66 ± 0.61	4.83 ± 0.64	4.36 ± 0.16
Vanillic acid	ND	ND	11.24 ± 0.62	16.08 ± 2.66	15.80 ± 2.44	12.71 ± 4.60
Trans-ferullic acid	ND	ND	ND	2.96 ± 0.03	3.02 ± 0.03	3.07 ± 0.09
Rutin	ND	ND	ND	20.41 ± 1.23	12.56 ± 0.39	9.60 ± 2.71
Xanthone	ND	9.18 ± 0.19	9.36 ± 1.24	ND	ND	ND
α-mangostin	24.28 ± 1.91*	252.95 ± 14.72	662.35 ± 7.53	463.73 ± 22.34	4.34 ± 0.39	4.65 ± 0.04
γ-mangostin	21.23 ± 1.43	117.37 ± 16.99	191.42 ± 5.49	148.25 ± 1.44	14.39 ± 0.40	17.95 ± 2.81

Values are means ± standard deviations (SD) from triplicate determinations (n = 3). ND: not detected.

($p < 0.05$). The order of reducing power corresponded with the total phenolic content of the extract shown in Figure 1(a). For DPPH• scavenging activity, the EC_{50} value was reported. PG, glycerol, and water were categorised in the group that provided the extracts with the highest DPPH• scavenging activity; whereas, ethanol, VCO, and hexane yielded extracts with lower activity. The difference in DPPH• scavenging activity among extracts was probably due to the extraction capacity of each solvent (Zarena and Udaya Sankar, 2009b). From the phenolic profiles of MP extracts, different types and quantities of phenolic compounds varied amongst the solvents tested (Figure 2 and Table 1). Tjahjani *et al.* (2014) reported that α-mangostin, non-polar phenolic compounds, had relatively poor DPPH• scavenging activity. The PG extract contained both polar and non-polar phenolic compounds. Thus, the PG extract had stronger DPPH• scavenging activity than extracts generated by ethanol, VCO, and hexane. These extracts mainly contained α-mangostin and γ-mangostin. However, standard α-tocopherol was the most powerful at scavenging DPPH• (EC_{50} of α-tocopherol = 22.86 µg/mL).

H_2O_2 scavenging activities of MP extracts were also affected by solvents used (Table 2). Normally, the decomposition of H_2O_2 can generate hydroxyl radicals that can facilitate lipid oxidation and DNA damage (Mukhopadhyay *et al.,* 2016). The lowest EC_{50} value for H_2O_2 scavenging activity was also found in extracts of PG, followed by those of ethanol/glycerol, water, VCO and hexane, respectively ($p < 0.05$). However, standard AA showed the most powerful scavenging H_2O_2 activity (EC_{50} of AA = 162.2 µg/mL).

In the literature, it is reported that the antioxidant capacity of phenolic compounds varied with the number and location of hydroxyl groups in the aromatic ring (Sroka and Cisowski, 2003). Thus, the excellent antioxidant activity of the PG extract could result from both polar and non-polar active constituents found in the extract.

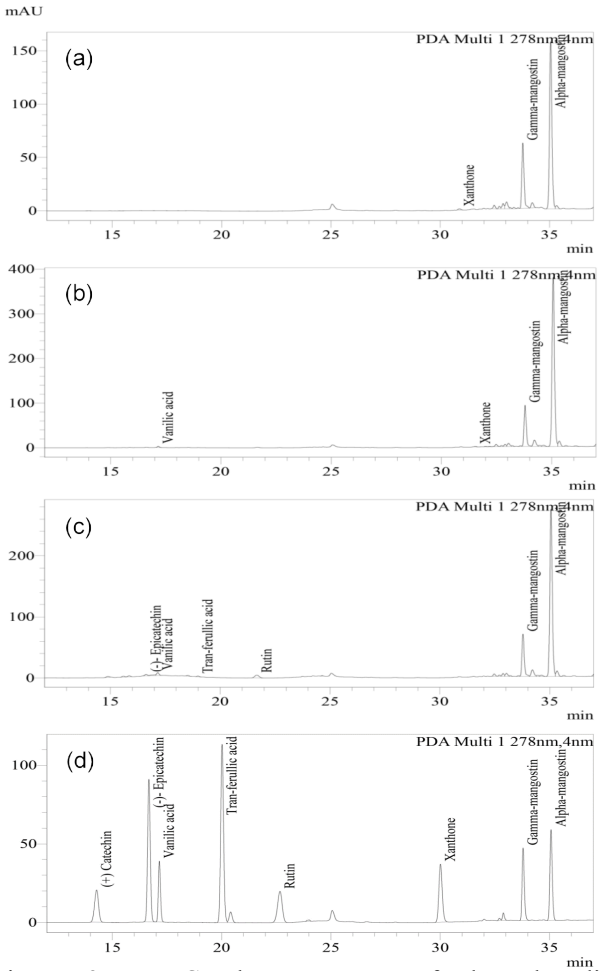

Figure 2. HPLC chromatograms of the phenolic compounds in MP extracts using different extraction media: VCO (a), ethanol (b) and PG (c) and the HPLC fingerprinting of the standard phenolic compounds (d).

Table 2. Bioactivities of MP extracts as affected by different solvents.

Bioactivity	EC_{50} values or MIC values (mg/mL)					
	Hexane	VCO	Ethanol	PG	Glycerol	Water
***In vitro* antioxidant activity**						
Reducing power (mg AAE /100 g)	5.90 ± 0.70[a]	47.90 ± 1.20[b]	78.90 ± 4.50[c]	209.70 ± 5.30[f]	192.70 ± 12.0[e]	153.60 ± 6.90[d]
DPPH assay[1]	114.33 ± 0.97[d]	6.22 ± 0.06[c]	3.31 ± 0.02[b]	1.21 ± 0.01[a]	1.48 ± 0.11[a]	1.62 ± 0.06[a]
H_2O_2 assay[2]	90.00 ± 1.60[e]	26.40 ± 0.40[d]	8.10 ± 0.10[b]	4.90 ± 0.40[a]	8.30 ± 0.30[b]	11.80 ± 0.70[c]
Antibacterial activity						
E. coli[3]	25	3.12	3.12	1.56	12.50	12.50
S. aureus[4]	25	1.56	6.25	3.12	25	25
***In vitro* antidiabetic activity**						
α-amylase[5]	101.16 ± 10.60[e]	18.24 ± 0.48[b]	3.16 ± 0.10[a]	2.75 ± 0.14[a]	28.84 ± 0.30[c]	46.69 ± 0.70[d]
α-glucosidase[6]	45.98 ± 4.68[b]	0.96 ± 0.02[a]	1.05 ± 0.14[a]	0.36 ± 0.04[a]	1.05 ± 0.05[a]	2.11 ± 0.04[a]

Values are means ± standard deviations (SD) from triplicate determinations ($n = 3$). Different letters within the same row indicate significant differences ($p < 0.05$). [1]EC_{50} value for DPPH-radical scavenging activity of α-tocopherol was 22.86 µg/mL. [2]EC_{50} value for H_2O_2 scavenging activity of ascorbic acid was 162.2 µg/mL. [3]MIC value against *E. coli* by ampicillin was 4 µg/mL. [4]MIC value against *S. aureus* by ampicillin was 4 µg/mL. [5]EC_{50} value for α-amylase inhibitory activity of acarbose was 5.84 µg/mL. [6]EC_{50} value for α-glucosidase inhibitory activity of acarbose was 667 µg/mL.

Antibacterial activity

The inhibitory effects of MP extracts, with various types of solvents, on the Gram-negative bacterium *E. coli* and Gram-positive bacterium *S. aureus* are summarised in Table 2. The type of solvent significantly affected the MIC values. MP extracts with PG and VCO as solvents had greater inhibitory effects against *E. coli* and *S. aureus* than the conventional solvent, ethanol, while hexane, water, and glycerol affected the bacteria only slightly. Based on the phenolic profiles presented in Table 1, higher α-mangostin and γ-mangostin contents in the ethanol extract did not always reflect the greatest ability to inhibit bacterial growth; although the presence of phenolic substances is believed to be responsible for the ability of the antimicrobial activity (Suksamrarn *et al.,* 2002; Pothitirat *et al.,* 2009). Antibacterial activity of MP extracts was dependent not only on the phenolics quantities but also on the phenolic profiles.

The VCO extracts had lower amounts of TPC, TFC (Figure 1), γ-mangostin and α-mangostin (Table 2) than the PG extracts, but VCO extracts showed similar inhibitory effects on *S. aureus* and *E. coli*. Some compounds found in VCO may be responsible for the synergistic effects with active compounds in MP, thus enhancing the suppression of microbial growth. The main compounds found in VCO are monolaurin and lauric acid, which have antibacterial effects against foodborne pathogens like *Listeria monocytogenes*, enterotoxigenic *E. coli* and *S. aureus* (Petschow *et al.,* 1998; Loung *et al.,* 2014). Both PG and monolaurin are non-ionic surfactants or amphiphiles having both hydrophilic and lipophilic properties (Widiyarti *et al.,* 2009). Therefore, they can delay the growth of both Gram-positive and Gram-negative bacteria. Gram-negative bacteria have thicker lipopolysaccaride layers and lauric acid and monolaurin can easily penetrate the membrane. In Gram-positive bacteria, although the membrane contains a very small amount of fat, but monolaurin, as a surfactant, can damage the bacterial cell membrane permeability barrier, causing the membrane to lyse and thus inhibit bacterial growth (Loung *et al.,* 2014). However, ampicillin showed superior antimicrobial activity to all extracts. MIC value of ampicillin against both *E. coli* and *S. aureus* was 4 µg/mL.

In vitro antidiabetic activity

Prevention of food carbohydrate absorption by inhibiting α-amylase and α-glucosidase is one of the proposed mechanisms for the treatment of diabetes (Conforti *et al.,* 2005). Synthetic acarbose has recently been used as a competitive inhibitor of these enzymes, but side effects e.g. abdominal pain and diarrhoea are frequently experienced (Supkamonseni

et al., 2014). Developing antidiabetic drugs that are efficacious and have minimal side effects is of a pressing need. To date, there is continuous discovery of phenolic compounds derived from herbal plants sources with potent antidiabetic activities (Arif *et al.,* 2014). Adnyana *et al.* (2016) reported that MP extracts contained phytochemicals that possess α-amylase inhibitory activity. Herein, α-amylase and α-glucosidase inhibitory activities of MP extracts with different solvents were tested. Linear trends in inhibition of α-amylase (Figure 3a) and α-glucosidase (Figure 3b) were observed in all extracts. Results suggested that α-amylase and α-glucosidase inhibitory activities of all MP extracts were concentration-dependent. The EC_{50} values for α-amylase inhibitory activity are also reported in Table 2. The EC_{50} values of MP extracts obtained by PG, ethanol, VCO, glycerol, water, and hexane extraction were 2.75 (R^2 = 0.975), 3.16 (R^2 = 0.988), 18.24 (R^2 = 0.970), 28.84 (R^2 = 0.983), 46.69 (R^2 = 0.960) and 101.16 (R^2 = 0.986) mg/mL, respectively (Table 2). Thus, PG extracts showed the highest α-amylase inhibition. The EC_{50} value of the standard drug acarbose was 5.84 μg/mL. The results also suggested that 471 and 3123 μg of crude MP extract generated by PG and VCO equalised to 1.0 μg of pure standard drug acarbose for α-amylase inhibition activity.

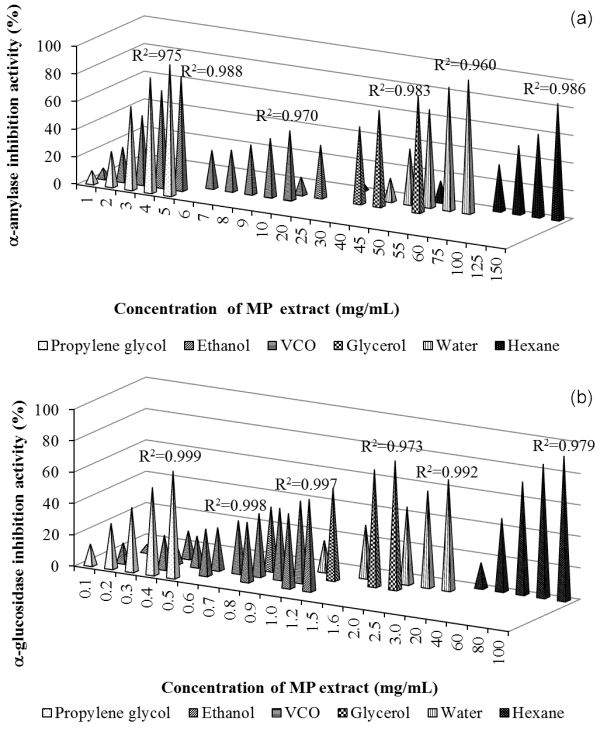

Figure 3. The inhibitory activity of MP extracts against α-amylase (a) and α-glucosidase (b).

The EC_{50} values for α-glucosidase inhibition of the standard drug acarbose and the MP extracted by PG, VCO, ethanol, and glycerol were 0.67 (R^2 = 0.998), 0.36 (R^2 = 0.999), 0.96 (R^2 = 0.998), 1.05 (R^2 = 0.997) and 1.05 (R^2 = 0.973) mg/mL, respectively (Table 2). From the Table, it is apparent that 0.5 and 1.4 μg of crude MP extracted using PG and VCO equalised to 1.0 μg of pure standard drug acarbose for α-glucosidase inhibition. In other words, PG extract exhibited 2-fold higher α-glucosidase inhibition than acarbose, whereas the VCO extract showed a lower activity than acarbose against α-glucosidase. In the present work, we demonstrated that the phenolic compounds in MP extracts prepared by PG, exhibited superior antidiabetic potential, relative to other extracts, by inhibition of both α-amylase and α-glucosidase. The inhibitory activity may be due to the presence of active phenolic profiles including, α-mangostin, γ-mangostin, (-) epicatechin, vanillic acid trans-ferullic, and rutin in the extract.

Conclusion

The present work highlighted the potential of bio-based solvents for the extraction of phenolic compounds from MP. Six extraction methods were performed using various solvents with different polarities. In conclusion, the TPC, TFC, phenolic profiles, antioxidant activity, antibacterial activity, and antidiabetic activity were affected by the type of solvent employed. The major phenolic compounds in all extracts were α-mangostin and γ-mangostin. The extraction using PG recovered both polar and non-polar phenolic compounds, and the extract showed superior *in vitro* antioxidant activity, antibacterial activity, and antidiabetic activity relative to the other extracts. Extraction of MP using VCO could enhance the antimicrobial activity of the extract. Therefore, bio-based solvents, particularly PG and VCO, are suitable for the extraction of phenolic compounds from MP. The resulting extracts can potentially be used as functional ingredients in food, cosmetic, and pharmaceutical applications.

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