Universiti Malaysia Terengganu Journal of Undergraduate Research *eISSN: 2637-1138*
Volume 4 Number 1, Januari 2022: 89-98

PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF FRUIT EXTRACTS OF *Momordica charantia* (var. charantia and var muricata)

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Abstract: *Momordica charantia,* commonly known as bitter gourd or bitter melon, is native to tropical and subtropical Africa and Asia. It is mainly used in traditional medicine for the treatment of various illnesses. This study was carried out to evaluate the antibacterial and antioxidant activity of two variations of fruits of *M. charantia* (var charantia and var muricata). Both samples were extracted with methanol and the crude extracts were subjected to phytochemical screening. Phytochemical screening revealed the presence of alkaloid, flavonoid, steroid, tannins and terpenoid in var charantia crude extract, whereas var muricata contained alkaloid, flavonoid, tannins and terpenoid. The susceptibility bacteria tests were performed by the disc diffusion method using six bacteria targets, with three strains of gram positive (*Bacillus cereus*, *Micrococcus luteus*, *Streptococcus aureus) and* three strains of gram negative (*Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa).* Crude extract of var muricata showed medium antibacterial potential against *S. Aureus* and *P. Aeruginosa*, while var charantia showed medium antibacterial potential against *E. coli* with an inhibition zone between (10-15 mm). Both extracts of *M. charantia* showed the lowest total antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity.

Keywords: M*omordica charantia*, antibacterial, antioxidant, DPPH.

Introduction

Momordica charantia, also known as bitter melon, karela, balsam pear, or bitter gourd, belongs to the family of Cucurbitacea, (Joseph & Jini, 2013). In Malaysia, this plant is also known as peria. *Momordica charantia* plant is grown in the tropical and subtropical regions of the world (Perumal *et al.,* 2015) and used traditionally for treating diabetes-related conditions amongst the indigenous populations of Asia, South America, India, the Caribbean and East Africa (Joseph & Jini, 2013).

A previous scientific study revealed that *M. charantia* extract might lower the blood glucose level of the diabetic rats (Perumal *et al*., 2015), lowers plasma lipid profile (Matsui *et al.,* 2013), and improve insulin resistance (Shih *et al.,* 2009), improved antioxidant status. In addition, supplementation of fresh ucche (*M. charantia* L var. muricata Willd) prevented the oxidative stresses, improved antioxidant enzyme function, and reduced hepatic inflammatory cell infiltration, ion deposition and fibrosis in the liver of CCl_4 treated rats (Sagor *et al.,* 2015). Several bioactive secondary metabolites were isolated from various parts of *M. charantia* plants, including phenolic and flavonoid compounds (Horax & Hettiarachchy, 2005), cucurbitane type triterpenoids (Pitipanapong *et al.,* 2007; Chen *et al.,* 2009; Nakamura *et al.,* 2006), cucurbitane type triterpene Glycoside (Nhiem *et al.,* 2010; Harinantenaina, 2006; Murakami *et al.,* 2001; Cao *et al.,* 2013), oleanane type triterpene saponins (Liu *et al.,* 2009) and peptides (He *et al.,* 2013). *M. charantia* illustrates broad diversity where the fruit morphology varies significantly in color, size and characteristics. As shown in Table 1, var charantia (VC) cultivars bear large fusiform

fruit while var muricata (VM) ecotypes in small round fruit (Bahera *et al.,* 2007). This was supported by Gaikwad *et al.* (2008), where both VC and VM are genotypically different because both variations were drawn from distinctly different ecosystems and are morphologically dissimilar.

Table 1: Description of variation *M.Charantia*

		
Scientific name	*Momordica charantia*	*Momordica charantia*
Variety	Var. charantia	Var. muricata
Characteristic	large fruits with fusiform shape	small and round fruit

This study aimed to screen the phytochemical constituents, determine the total phenolic and flavonoid contents, and evaluate the antibacterial and antioxidant properties of two variations of fruit extracts of *M. charantia,* var charantia (VC) and var muricata (VM).

Materials and Methods

Sample Preparation

Fruits of VC and VM were obtained from a local vegetable market in Batu Enam, Kuala Terengganu, Terengganu, in July 2018. The fruit sample was cut into small pieces and dried in the oven at 38 ^{O}C temperature for two days. After the dried process, the fruit samples were ground into powder form.

Extraction of Sample

The powdered fruit of VC (45.96 g) and VM (35.79 g) was extracted with methanol for 24 hours. Both each extract was decanted and filtered through Whatman filter paper no. 1. The extraction process was repeated three times. The extracts were then evaporated under reduced pressure to give 756 mg of VC (756 mg) and 565 mg of VM (565 mg) crude extracts, respectively. The extracts were kept in a fridge at 4 °C for further use.

Phytochemical Screening

Alkaloid Test

Extracts were dissolved individually in dilute hydrochloric acid and filtered. Filtrates were then treated with Mayer's reagent (potassium Mercuric iodide). The formation of a yellow precipitate indicates the presence of alkaloids (Tiwari *et al.,* 2011).

Flavanoid Test

Alkaline Reagent Test: Extract were treated with a few drops of sodium hydroxide solution. The formation of intense yellow colour, which becomes colourless with the addition of dilute acid, indicates the presence of flavonoids (Tiwari *et al.,* 2011).

Steroid Test (Liebermann-Burchard's test)

0.5 mL extract was dissolved in 2.0 mL acetic anhydride and cooled in ice. Then, the 1.0 mL concentrated sulfuric acid ($H_2SO_{4)}$ was added, and the blue-green ring was considered positive for the presence of steroids (Singh *et al.,* 2012).

Tannins Test

In 1.0 mL extract, an equal volume of bromine water was added, and the formation of greenish-black (reddish-brown) precipitate indicated the presence of tannins (Singh *et al.,* 2012).

Terpenoid Test :Salkowski test

Five mL of each extract was mixed with 2 mL chloroform and 3 mL concentrated H_2SO_4 were carefully added to form a layer. A reddish-brown coloration of the interface was formed to show a positive result for the presence of terpenoids (Sheel *et al.,* 2014)

Antibacterial Test

The antibacterial activity of each extract was carried out using the disk diffusion method (Andriani *et al.,* 2015). Both crude extracts of *M. charantia* were tested against *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Micrococcus luteus, Streptococcus aureus,* and *Bacillus cereus*. Ampicillin, penicillin, gentamycin, and tetracycline were used as standards. All the bacteria were obtained from Hospital Sultanah Zahirah Kuala Terengganu. Firstly, all bacteria stocks were subcultured for 24 h before they were used for antibacterial activity. After 24 h, the six types of target bacteria suspension (the concentration of the bacteria is 0.5 McFarland standards) were spread evenly using a cotton swab onto the Mueller Hilton agar (MHA). Separately, each extract was subjected to serial dilution by using dimethyl sulphoxide (DMSO) as a solvent to give 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL and 0.625 mg/mL sample concentrations. Samples (20 µL) were loaded onto each paper disk. Furthermore, the paper disk content of the sample was put on the surface of MHA. All plates were inverted and incubated for 24 h at 37 ^{O}C. The diameter for the inhibition zone (mm) of each sample was measured.

Antioxidant test

Free radical scavenging activity of methanol extracts of fruits of *M. charanthia*, (VC and VM) were measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) (Andriani *et al.,* 2018) with slight modification. Quercetin was used as a positive control and DMSO as negative control. Crude extract of *M. charantia* was prepared in varying concentrations by two-fold serial dilution in DMSO with concentrations 10, 5, 2.5, 1.25 and 0.625 mg/mL in 96 well plates. In addition, 200 µL of methanolic DPPH solution (6 x 10^5 M) were added to all well and the mixture was incubated for 30 minutes at room temperature. The absorbance was measured at 517 nm using Elisa reader (Multiskan ascent, Thermo Electron Corporation). Free radical scavenging activity was determined according to the equation:

$$\text{Free radical scavenging activity (\%)} = \frac{\text{Ac-As}}{\text{Ac}} \text{x100\%}$$

where,

As = absorbance of sample

Ac = absorbance of negative control

Total Phenolic Content

The total phenolic content of each extract was determined by the Folin–Ciocalteu (FC) method (Zhang *et al.,* 2018) with slight modification. Briefly, 125 µL of the diluted of each extract (VC and VM) were mixed with 0.5 mL of distilled water. Then, 125 µL of the Folin–Ciocalteu reagent were mixed into the solution. After six minutes, 1.25 mL of a 7% aqueous Na_2CO_3 solution was added to the mixture and allowed to stand for 90 minutes. The absorption was measured at 760 nm using Shimadzu UV-1800 against water as a blank. Gallic acid was used as standard. The amount of total phenolics is expressed as gallic acid equivalents (GAE, mg gallic acid/g sample) through the calibration curve of gallic acid.

Total Flavonoid Content

Total flavonoid content was determined using colorimetric method (Taroreh *et al.,* 2016) with slight modification. Briefly, 0.25 mL of each extract (VC and VM) or quercetin standard solution were mixed with 1.25 mL of distilled water, followed by 75 µL of 5% $NaNO_2$ solution.

After 6 minutes, 150 µL of a 10% $AlCl_3.6H_2O$ solution was added (allowed to stand for 5 min). Then, 0.5 mL of 1M NaOH was added. Finally, the mixture was added with distilled water (brought to 2.5 mL). The absorbance was measured against distilled water as blank a 510 nm using a spectrophotometer compared with the quercetin as a standard.

Results and Discussion

Phytochemical Screening

Phytochemical screening was performed using standard protocols (Tiwari *et al.,* 2011, Singh *et al.,* 2012, Sheel *et al.,* 2014) to identify the presence of alkaloids, flavonoids, steroids, tannins and terpenoids. Table 1 below shows the summary of phytochemical screening. The extract of VC showed the presence of alkaloids, flavonoids, steroids, tannin and terpenoid, whereas the VM showed the presence of alkaloids, flavonoids, tannin and terpenoid.

Table 2: Phytochemical screening in fruit extracts of *M. charantia* (VM and VC)

Phytochemical Test	VC	VM
Alkaloids	+	+
Flavonoid	+	+
Steroid	+	-
Tannins	+	+
Terpenoid	+	+

+ = presence; - = absence

Antibacterial Activities

The present study analyzed the antimicrobial activity of MC plants used as medicinal plants in local traditional medicine. Very little information is available concerning the antimicrobial activity of the studied plant variations. Table 3 shows that both samples exhibited antibacterial activity at varying minimum inhibition concentrations (MIC). It is found that VM possessed an excellent antibacterial activity against Gram positive and Gram negative bacteria tested compared to the VC. The extract of VM exhibited a medium antibacterial activity towards Gram positive bacteria (*S. aureus*) and Gram negative bacteria (*P. aeruginosa*) with the highest MIC value obtained at 0.625 mg/mL with inhibition zone (10-15mm). The extract of VM also showed weak antibacterial activity against *K. pneumonia, M. luteus* and *E. coli,* with a MIC value of 0.625 mg/mL against *M. luteus* and *E. coli.* Moreover, *P. aeruginosa* was found to be the most sensitive strain once compared to other strains, while *B. cereus* was found to be the most resistant to all plant parts extracts. Indeed, the difference in sensitivity between Gram positive and Gram negative bacteria can be attributed to morphological differences in these microorganisms, most notably differences in cell wall permeability (Bereksi *et al.,* 2018). The extract of VC did not possess antibacterial activity against Gram positive bacteria tested. However, it revealed medium antibacterial activity against Gram positive bacteria tested *E. coli* with MIC also 0.625 mg/mL. In addition, both extracts showed no antibacterial activity against gram positive bacteria tested *B. cereus.*

Table 3: Antibacterial activities of fruits of *M. charantia* (VC and VM)

Types of bacteria	Concentration (mg/L)	Inhibition Zone	
		VC	VM
Klebsiella pneumonia	10	-	8 mm
	5	-	8 mm
	2.5	-	7 mm
	1.25	-	-
	0.625	-	-
Micrococcus luteus	10	-	8 mm
	5	-	8 mm
	2.5	-	7 mm
	1.25	-	9 mm
	0.625	-	8 mm
Streptococcus aureus	10	9 mm	14 mm
	5	8 mm	12 mm
	2.5	7 mm	10 mm
	1.25	9 mm	9 mm
	0.625	9 mm	8 mm
Pseudomonas aeruginosa	10	9 mm	15 mm
	5	8 mm	14 mm
	2.5	8 mm	10 mm
	1.25	7 mm	10 mm
	0.625	7 mm	10mm
Escherichia coli	10	9 mm	9 mm
	5	8 mm	8 mm
	2.5	7 mm	7 mm
	1.25	9 mm	8 mm
	0.625	11 mm	8 mm
Bacillus cereus	10	-	-
	5	-	-
	2.5	-	-
	1.25	-	-
	0.625	-	-

**(-) No activity, (7-10mm) Weak activity, (11-15mm) Good activity, (≥15mm)Strong activity

Determination of Antioxidant Activities

The DPPH free radical scavenging method is when a molecule or antioxidant with weak A-H bonding will react with a stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl, max=517 nm), causing discoloration of the molecule. From the analysis of Figure 1, we can observe that the antioxidant activity of VC and VM was determined using the quantitative assay, where the graph of antioxidant percentage (%) against the concentration of the sample (mg/mL) was observed according to the IC_{50} value. A sample extract of *M. charantia* had less than 50% antioxidants and did not achieve IC_{50} value by using DPPH free radical scavenging methods. The scavenging of the stable DPPH radical model is a commonly used technique for quickly screening antioxidant properties using a spectrophotometer. When an antioxidant molecule interacts with a DPPH radical, the absorbance at 517 nm decreases. This is because antioxidants scavenge the radical by donating hydrogen to form the reduced form (DPPH-H). This property is also visible as the color changes from purple to yellow. The more active the antioxidant compound, the faster the absorbance decreases (Anjamma and Bhavani, 2018). The greater the percentage of scavenging, the greater the hydroxyl radical scavenging activity, indicating that VC extract had a higher hydroxyl radical scavenging activity than VM extract. The effective sample concentration needed to scavenge 50% of the DPPH free radicals was designated IC_{50} (Rani *et al.,* 2018). As a result, the lower the concentration needed to scavenge 50% DPPH free radicals, the higher the antioxidant activity. Therefore, the hydroxyl radical scavenging activity of all the extracts was increased. However, it performed significantly worse than the positive control quercetin.

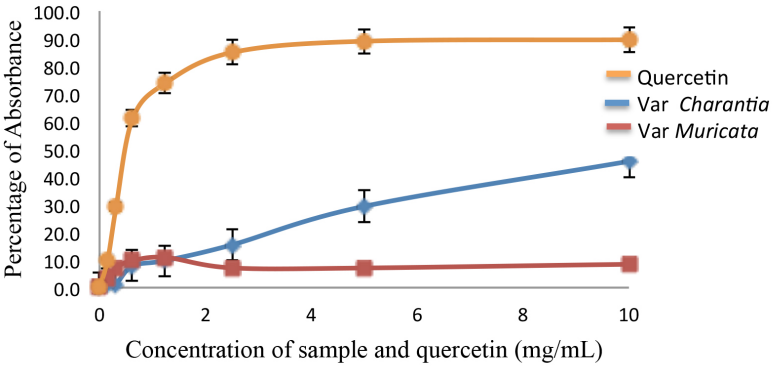

Figure 1: Graph of DPPH free radical scavenging activity

Determination of the Total Phenolic Content (TPC)

Phenolic compounds are widespread in the plant kingdom, acting as antioxidants and free radical scavengers. Gallic acid was used as a standard in determining the total flavonoid compound present in samples. The standard was introduced to UV – Vis spectrophotometer and was measured at 760 nm to get the maximum wavelength. Extract of (VC) shows the highest phenolic content when at concentration 1.0 mg, the absorbance is 0.446 compared to VM with the absorbance of 0.217 (Figure 2). The phytoconstituents, antioxidant potential, total phenolics, and flavonoid content of MC fruit extracts were investigated. The phytochemical screening was done to determine which chemical compound groups were present in the extracts. According to the phytochemical profile, the plant extract contains molecules with high technical potential for producing new drugs. Antioxidant vitamins, phenolics, and tannins can contribute to the antioxidant activity of these

traditional medicinal plants. Antioxidant activity is frequently linked to phenolics, especially flavonoids (McCune *et al.,* 2002). As shown in Fig. 2, VC samples had higher TPC than VM extract. Although TPC in VC was slightly higher than VM, no significant difference was observed. In the determination of total phenolics and flavonoids, the results showed that the VC extract was better than VM extract, phenolic compounds whose good polarity and solubility may explain phenolic compounds extracted from plants (Chahar and Sharma 2017). The phenolic compounds are abundant in MC because of their health-related properties, higher concentrations of phenolic compounds showed more potent bioactivities in food items **(**Gao *et al.,* 2019**).** Based on this association, it is reasonable to conclude that phenolic compounds were the primary contributors to increased total antioxidant activity and ferric reducing ability in both varieties of fruit extracts.

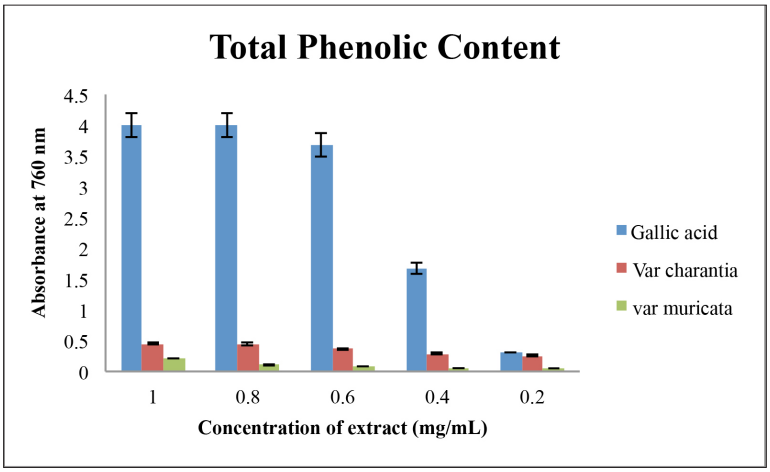

Figure 2. Total phenolic content of gallic acid and sample

Determination of the Total Flavonoid Content (TFC) in samples

Quercetin was used as a standard in determining the total flavonoid compound present in samples. The standard was introduced to UV – Vis spectrophotometer and was measured at 510 nm to get the maximum wavelength. The production quercetin was plotted and calculated using Microsoft Excel (Figure 3) using the equation from the graph, y = 0.0387x + 0.0151 with the value of regression, $R^2 = 0.9893$. Extract of VM shows the highest flavonoid content where at concentration 1.0 mg the absorbance is 0.0471 compared to VC with the absorbance of 0.0271. The production of VC also were plotted Figure 3 using the obtain equation from graph, y = 0.0242x + 0.0034 with the value of regression, $R^2 = 0.9969$ while for VM the equation from graph, y 0.0357x + 0.011 with the value of regression, $R^2 = 0.9975$.

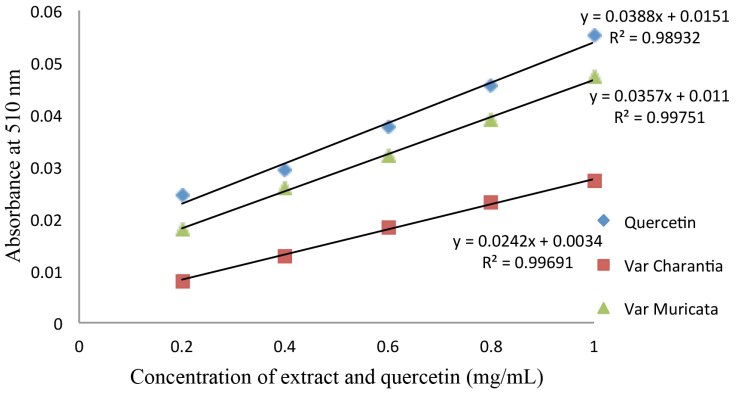

Figure 3: Calibration curve of quercetin and sample

Conclusion

The phytochemicals screening shows the presence of alkaloids, flavonoids, tannins and terpenoids in both variations of fruit extracts of *M. charantia* (VC and VM). Extracts of *M. charantia* in both variation (VC and VM) is a good source of phenolic compounds which possess potent antioxidant activity. Flavonoids and tannins in both extracts demonstrated that bitter melon is an important source of phenolic compounds with strong antioxidant activity. Furthermore, both variation of *M. charantia* is a suitable and environmentally that could enhance the total phenolic compounds extracted from this plant. Whereas steroids are only present in var *charantia*. Based on the results of the antibacterial assay, *var muricata* exhibited antibacterial activity for five types of bacteria (*E. coli, K. pneumonia, P. aeruginosa, M. luteus, S. aureus*). In comparison, var *charantia* only exhibited antibacterial activity for three types of bacteria (*S. aureus, P. aeruginosa, E. coli)*. Extract of VC shows the highest phenolic content at a concentration of 1.0 mg, and the absorbance is 0.446 compared to VM with an absorbance of 0.217. Whereas VM extract shows to have the highest flavonoid content compared to VC.

Acknowledgements

The authors thank the Universiti Malaysia Terengganu for supporting this research.

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